PRECLINICAL STUDY OF SIDDHA DRUG VENKARA CHUNNAM'S LITHOTRIPTIC, DIURETIC AND ANTI SPASMODIC ACTIVITIES

Dissertation submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfillment of the requirements

for the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

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JULY 2019

GOVT. SIDDHA MEDICAL COLLEGE
PALAYAMKOTTAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre-clinical study of Siddha drug VENKARA CHUNNAM for its lithotriptic, diuretic, antispasmodic activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Antony Duraichi M.D(s)., Lecturer Grade II,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Palayamkottai and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ABBREVIATIONS

VC	-	Venkara Chunnam
ALT	-	Alanineamino transferase
ANOVA	-	Analysis of Variance
CCDs	-	Charge coupled devices
CPD	-	Calculi Producing Diet
FT-IR	-	Fourier Transform - Infra red Spectroscopy
SGOT	-	Serum Glutamate oxalo acetate transaminase
SGPT	-	Serum Glutamate pyruvate transaminase
IAEC	-	Institutional Animal Ethical Committee
ICP-OES	-	Inductively Coupled Plasma Optical Emission Spectroscopy
PCV	-	Packed cell volume
RBC	-	Red blood corpuscles
TLC	-	Thin layer chromatography
SEM	-	Scanning Electron Microscope
GC	-	Gaschromatography
WBC	-	White blood corpuscles
CRB	-	Calcium Reserve Body
No	-	Number
Mg	-	Milligram
Kg	-	Kilogram
LD_{50}	-	Lethal $Dose_{50}$
p.o	-	peros
ML	-	Milliliter
R&D	-	Research and Development
EDTA	-	Ethylene Diamine Tetra Acetic Acid

M	-	Male
G	-	Gram
NOAEL	-	No-Observed-Adverse-Effect-Level
MLD	-	Minimum Lethal Dose
MTD	-	Maximum Tolerated Dose
OECD	-	Organisation of Economic Co- operation and Development
CPCSEA	-	Committee for the Purpose of Control and Supervision of Experiments on Animals

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Expiry Date : Nil

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TAMIL NAME	ENGLISH NAME	CHEMICAL NAME
VENGARAM	BORAX	*SODIUM BI BORATE*

Date : 4.7.18

Station: Palayamkottai

4/7/18.

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TAMIL NAME	BOTANICAL NAME	FAMILY	PART USED
Uppilangodi	*Pentatropis microphylla Roth ex Schult.*	Asclepediaceae	Whole plant
Murungai pattai	*Moringa oliefera Lam*	Moringaceae	Bark

Date : 4.7.18

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for participating as ~~Resource~~ ~~Person~~ / Delegate in the XXIII Workshop on

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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY IN SIDDHA DRUG FORMULATION KAALAKODI RASAM

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ABSTRACT
Siddha system is the ancient system of heeling and it's based on combination of medical practices and preventive methods, as well as alchemy and mysticism. Herbo metallic preparations play an important role in traditional system of medicine. One such effective & safe herbo metallic formulation is kaalakodi rasam (KKR). As indicated in Siddha literature KKR is a herbo metallic formulation treated for osteoarthritis. **Aim:** To study of analgesic and anti-inflammatory activity in the siddha drug formulation Kaalakodi rasam. **Materials and methods:** The analgesic and anti-inflammation activities evaluation were done by Acetic acid Induced Writhing Test method in Swiss albino mice and Carrageenan induced acute hind paw oedema method on Wister albino rats, respectively. Doses of different proportions 100mg, 200mg of KKR powder suspension were given to the animals for a stipulated period of time. **Results and conclusion**: KKR having significant analgesic activity against the acetic acid-induced writhing in mice and anti-inflammatory activity against the carrageenan -induced paw oedema in rats.

KEYWORDS: Kaalakodi rasam, analgesic and anti-inflammatory study, siddha, osteoarthritis.

INTRODUCTION
Siddha system is the ancient system of heeling and it's based on combination of medical practices and preventive methods, as well as alchemy and mysticism. Other system of medicines is gives priority to herbal preparation for treating disease but our Siddha medicines preparation we are using combination of plants, metals, and minerals. Herbo-metallic preparations play an important role in traditional system of medicine. While such preparation are held to be safe, effective in small doses, when prepared and used following specific guidelines of Siddha text.[1]

One such effective & safe herbo-metallic formulation is kaalakodi rasam. In Siddha literature KKR is a herbo-metallic formulation treated for osteoarthritis. Osteoarthritis (OA) is a disease of cartilage degradation, which results pain in major joints, especially in knee joint. Globally OA ranks eighth in all diseases and covers around 15% proportions among all musculoskeletal problems. Clinical symptoms and radio-diagnosis are the basis of diagnosis used for OA characterization. India has higher proliferative rate of OA among world and expected to be at top rank in chronic diseases till 2025.[2]

AIM AND OBJECTIVE
To study of analgesic and anti-inflammatory activity in the siddha drug formulation Kaalakodi rasam.

MATERIAL AND METHODS
Collections of drugs
The drugs were collected from reputed raw drug shop in Madurai and Nagerkoil. These drugs were analysed and authenticated by Government Siddha Medical College, Palayamkottai.

Method of preparation of KKR
The KKR drug was prepared under siddha literature Chikitcha Rathnam Deepam Ennum Vaidhya Chinthamani by kannusaamy pillai. First make gajili with Purified rasam (Hydragirum) & ganthagam (sulphur). Then fried thalagam (Trishulphate of arsenic), lingam (Red sulphide of mercury), naabi (aconitum napellus root), naervalam (croton tiglum seeds), sukku (zingiber officinale root), arisithipilli (piper longum fruit), milagu (piper nigrum fruit), kadukai (terminalia chebula fruit) thaanrikaai (terminalia bellirica fruit), nelli (phyllanthus emblica fruit), nerunjil (tribulus terres fruit) are in a pan and make it as powder. Then mix with Alli kilangu juice (nymphaea nouchalli fruit) and grind in a kalvam for 3 samam (9hrs). Then making pills as 130 mg

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SINGLE HERBALS USED IN SIDDHA SYSTEM FOR ENT DISORDERS

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ABSTRACT

Head is the most important part in our body. Our siddha system is based on five elements theory. Ear nose throat are parts of five senses which are correlated with those elements. ENT problems come under the section of head disease. Nowadays, ENT disorders affect in our day to day life. In this survey 4.31% of the rural populations of the district were having ENT ailments. The highest numbers rural populations (35.65%) were having ear diseases. It is the most important to cure and management the ENT disease and avoids further infections. There is lot of herbals described singly for the treatment for ENT. Plants used in Siddha system of medicine for ENT has been studied from various Siddha literatures and presented here under. The particulars of herbs used with its botanical name, vernacular name (Tamil), family, parts used, administration form and indication are tabulated. The most used family and part used for ENT disorders are charted. **Conclusion:** In this research finding, results explored as most used family is Lamaceae, Fabaceae, Apiaceae, Solanaceae, Zingiberaceae and part used were Leaves, fruits, rhizomes, flowers, seeds and barks. Therefore, Lamaceae family and leaves were high rank used in treating ENT disorders. This information provides immense potential for studying their activity for ENT both in pre-clinical and clinical stages, which lead to the preparation of useful pharmaceutical products.

KEYWORDS: ENT disorders, siddha, herbal, ear disorder, nose disorder, throat disorder.

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1. INTRODUCTION

Siddhars are men with supernatural power and Medical wisdom. All their doing were highly intertwined with nature. They have combined their Medical works with astrology, alchemy & philosophy. They were robust in the concept "Nature that causes diseases and it is again nature effects their cure" and it is therefore necessary that the physician should know the principles of nature.

According to the *Siddhars* concept

அண்டத்தில் உள்ளதே பிண்டம்
பிண்டத்தில் உள்ளதே அண்டம்

-சட்டைமுனி ஞானம்.

Siddhars were proficient with formulations that treat and prevent ailments. The formulations were either purely herbal or animal or metals or a combination of herbomineral or metals. Thus for preparing medicine, Siddhars solely depend on natural wealth.

The Siddha system based on three vital humours namely *vatham, pitham and kabam.* Siddha is the first system to emphasize on food habits. If human beings have any alteration in food habits, it will affect the vital elements of their body.

According to *Thiruvalluvar,*

மாறு பாடில்லாத உண்டி மறுத்துண்ணி
ஊறு பாடில்லை உயிர்க்கு.

\- *Thirukkural*

One of the *ancient siddhar Yugi* had classified the diseases into 4448 types. He had classified Urinary disorder into

1. *Neerinai Arukkal Noigal*
2. *Neerinai Perukkal Noigal*

Kalladaippu (renal stone) disease comes under *Neerinai Arukkal Noigal.*

In our siddha system Urolithiasis may be compared to *Kalladaippu* is the most common diseases of present society due to modern life style and abnormal diet habits.

Kalladaippu is a common disease of urinary tract which has the following symptoms, *Neer erichal* (burning micturation), urinary obstruction (*Neer adaippu*), low back ache, referred pain in genital organs and tip of penis, abnormal deposits in the urine. In modern science, these symptoms can be correlated with 'urolithiasis'.

'Urolithiasis is a common disorder estimated to occur in approximately 12% of population with a recurrence rate of 70% - 80% in male and 47% - 60% in female.

In India, 5% of people are seeing affected by kidney stones and 8-10% people who have life time risk of passing the kidney stone. The recurrence rate for urinary calculi is very high, approximately 50% among the Indian population. In 2013, Kidney stone prevalence is higher in men (66%) in compare to females and common with age group 31-40 years. During the last two decades the rate of renal stone recurrence was about 75%.

The prevalence figures of stone disease observed in developing country in tropical regions are similar to rates of western countries with incidence of renal colic particularly high in warm months. Ultimately, in our country, the climatic condition prevailing here plays a major role along with abnormal diet habits, insufficient water intake, and sedentary life style.

The efficacy of invasive therapies such as extra corporal shock-wave lithotripsy and ureteroscopy has been proven by several studies. However these techniques are not risk free and they are problematic and quite expensive and complication.

In our Siddha system the number of drugs has been prepared from the medicinal plants which are source of drugs for *Kalladaippu.*

Drug means any substance that when taken into a living organism may modify physiological system or pathological states. Siddhars were persons of highly cultured intellectual & spiritual faculties with a very vast boundless knowledge about medicine and alchemy.

A drug possessing high therapeutic index is said to be safe. Reaction to a drug in a subject may vary in accordance with various factors which includes anaphylaxis, idiosyncrasy or accidental overdosing. Variation of dose, duration alters the medicine and makes it a poison.

Dose and duration of administration of drug is much more important for clinical practice. It is the duty of a physician to ensure that the patient is receiving the correct dose and is harmless. Or else, the physician will be charged for his medical negligence under legal aspects.

According to *Anuboga vaithiya navaneetham, part 3 page no 25, 26* there was a preparation of *VENKARA CHUNNAM* which is indicated for *Kalladaippu*. I have selected the drug to evaluate for its Lithotriptic, Diuretic, and Anti-Spasmodic (smooth muscle relaxants) activities.

2. AIM AND OBJECTIVES

AIM:

The main aim of this dissertation work is to do a scientific validation of *VENKARA CHUNNAM* for its Lithotriptic, Diuretic, Anti-spasmodic activities in treating *Kalladaippu* (Urolithiasis) disease.

OBJECTIVES:

The main objective of the present study is safety and efficacy of the *VENKARA CHUNNAM* in the treatment of *kalladaippu*, the following methodology was adopted to evaluate the drug and its standardization studies.

1. Collection of literature evidence regarding the trial medicine.
2. Identification of the drugs in the *VENKARA CHUNNAM.*
3. Preparation of the trial medicine as per the text.
4. Physico – chemical analysis of the test drug.
5. Biochemical analysis of the test drug.
6. Phytochemical analysis of the test drug.
7. Instrumental analysis of the test drug.
8. Evaluation of acute and sub-acute toxicity of the test drug according to the OECD guidelines.
9. Evaluation of pharmacological activity of the test drug.
    - Lithotriptic activity
    - Diuretic activity
    - Anti - spasmodic activity
10. Antimicrobial activity of the test drug.

3. REVIEW OF LITERATURE

3.1 *VENKARAM* (BORAX)

3.1.1 GUNAPADAM ASPECT

Among minerals there is 25 karasaram. It is divided into 10 natural and 15 artificial. Venkaram belongs to Natural type.

SYNONYMS:

வெண்காரப்பேர்தனையே விளம்பக் கேளு
மேதினியோர் தங்களுக்கு உருக்கினமாகுஞ்
சங்காரம் போலவே சத்தெல்லாமாகும்
சத்தானப் பொரிகாரிக் குடோரியாகும்

Porikaram, Karam, Urukkinam, Danganam, Urukkumithiran, Thoomathaiyadakki.

அங்காரி டங்கணமுமா மடங்காத
துமத்தை அடங்கப் பண்ணி
பொங்காரி சரக்குக்கு மித்துரு வேயான
பொரிகாரி தனக்கிசைந்த பேருமாமே.

Urukkinam, Porikari, Kudori, Ankari, Danganam, Ponkari, Sarakku mithru

-bogar nikandu 1200 page.no – 5

OTHER NAMES

- *Karam, Pethagamani, Uruku Mithiran,Siripari, Porikaram, Urukinam, Santhani, Kudoori, Tanganam, Dhoomathai Adaki,*

  - Sattamuni Nigandu

- *Karam, Porikaram, Urukinam, Urukumitharan, Tanganam, Dhoomathai Adaki.*

  -Gunapadam Thathu Jeevam

- *Vedikaram, Kabeeram, Kabesam, Kudori, Kurinchanam*

  -Pachilai Mooligai Agarathi

- *Logasuthi Karakam, Thiravi, Thiravangam, Malatherasathin Sambaram,*

  -Nigandu Rathina Kaaram

PANCHA BOOTHAMSAM

Vengaram is vaayu boothamsam as declained through this song

அறிந்து கொள் வெடியுப்பும் சவுட்டினுப்பும்
அரகரா தேயுவென்றே அறியலாகும்
தெரிந்து பார் வெங்காரந் துருசி ரண்டும்
திறமான வாயுவென்ற செப்பலாகும்

-Bogar Kaarasaara Thurai

VERNACULAR NAMES:

Tamil	-	*Vengaram, Venkaram*
Sanskrit	-	*Tankana, Tunkana, Rasashodhan*
English	-	Borax, Tincal, Sodium Biborate, Sodium Borate,, Biboratc of Soda, tynkal, Biborate of sodium, Pyroborate sodium, Tetraborate sodium,
Hindi	-	*Tinkal, Tincal, Sohaga*
Gujarati	-	*Tankana, Khara*
Malayalam	-	*Pongaaram*
Telugu	-	*Veligaram, Elegaram*
Urdu	-	*Tankar, Suhaga*
Bengali&		
Punjabi	-	*Sohaga, Suhaga, Tinkar, Tinkal*
Arab	-	*Buraekes - Saghah*
Persi	-	*Tinkar - Tankar*
Tibetean	-	*Chusal*
Kanada	-	*Biligara*
Burma	-	*Lakhiya*
Malay	-	*Pijar, Palleri*

-The Wealth of India page-199.

ORIGIN AND OCCURRENCE:

Borax is obtained is California abundantly. It is in Tibet and Nepal. In India it is found in Ladakh and Puga valley of Kashmir. Tincal contains about 55% of borax. Naturally it is obtained along with sand and dust. The borax available in shops is not

pure. Hence four in parts of Hot water and a small amount of calcium carbonate (lime) are added to it, filtered, insolates and heated till the water evaporates completely. The salt so obtained is pure and can be used.

Leh in Ladakh district of Jammu and Kashmir

Surendranagar district of Gujarat

Nagpur and Jaipur districts of Rajasthan

UttarPradesh

California, USA and Kramer

Argentina, China, Peru and Chile

SearlesLake

SOURCE

Borax occurs as natural deposits. Tincal is the name given to crudeBorax. It is found in masses by evaporation of water, on shores of dried uplakes and also obtained from the mud of lakes.

COMPOSITION:

Na_2O	:	16.25%
B_20_3	:	36.60 %
H_20	:	47.24 %
Sodium	:	12.06 %
Boron	:	11.34 %
Hydrogen	:	05.29 %
Oxygen	:	71.32 %

SYNTHETIC PREPARATIONS (VAIPPU):

INGREDIENTS:

GROUP – A

1. Fuller's earth (*Pooneeru)* - 1.3 Litre (1 *Padi)*
2. Water - 10.4 Litre (*8 Padi)*

GROUP - B

1. Alum (*Cheenam*) - 3500 gm (*100 Palam)*
2. Potassium nitrate (*Vediuppu)* - 219 gm (6 ¼ *Palam*)
3. Milk spurge burnt ash - 1.3 Litre
4. Indian liquorice leaf juice - 1.3 Litre
5. Castor Oil

Fuller's earth is dissolved in water and filtered. Group – B drugs are added to the above filtrate and insolates. It becomes black. Then the order 3 drugs are added to it and heated with *Kamalakkini* for 96 minutes. Then it is placed in an earthen pot with wider mouth and insolates. At the end, the product is collected.

-Bogar 7000, Irandam kaandam.

CHARACTERS OF VENKARAM

- Clear, white and luster
- Dissolved in water, undissolved in alcohol.
- When kept in open air white colour layer formed in upper part of venkaram
- When venkaram is fried the water content lost, small hole obtained in fried venkaram.

SPECIALITIES:

காரமென் றிதற்குப்பேர் வந்த தேது
கட்டுமே அறுபத்து நாலு தாதும்
காரமென் றிதற்குப்பேர் வந்த தாலே
கடிசான வுபரசநூற் றிரண்டுஞ் சத்தாம்
காரமென் றிதற்குப்பேர் வந்த தென்றால்
கட்டாத சாரந்தானி தற்குள் கட்டும்
காரமென் றிதற்குப்பேர் வந்த தாலே
களங்குகுரு சிந்தூரத் தாதி காணே.

- It is very important drug for preparing *parpam, Chendooram* and *guru.*
- It had the capacity to make *Kattu* of 64 *Paadaanam*.
- Make 120 *Ubarasam* into *Satthu* medicine form.
- Make *karam* into *Kattu* medicine form.

-Bogar 7000 irandamaayiram.

ORGANOLEPTIC CHARACTERS:

Taste	-	Sweet with astringent
Potency *(Veeriyam*)	-	Heat (*Veppam)*
Biotransformation *(pirivu)*	-	Pungent *(Kaarpu)*

Sweet and astringent taste of *Venkaram* has earth, water and air elements.

ACTIONS:

Lithotriptic

Diuretic

Astringent

Antiseptic

- Indian Materia Medica

Internal

Refrigerant

Diuretic

Emmenogogue

Lithotriptic

Parturifaceient

External

Sedative

Alterative

Antiseptic

Astringent

- Gunapadam thaathu jeeva vagupu

GENERAL PROPERTIES:

‘சொறிபுடையெண் குன்மநமை சோரி யாசம்
பறிகிரகணி கல்லுனம் பன்னோய் - நெறியைத்
தடங்கணங்க பங்கிருமி சர்ப்பவிடஞ்சத்தி
உயிரிடங்கணங்க லக்கிற்போ மென்.

It relieves toad skin, carbuncle, 8 types of *gunmam,* itching, bleeding biles, renal calculi, dental disease, urinary tract infections*, kapam*, poison due to snake etc.

- Gunapadam thaathu jeeva vagupu

வெங்காரக் குணமிதென்று விதமுடனுரைக்கக் கேளாய்
சங்கார மாகுந்தோஷந் தன்னையே சங்கரிக்கு
முங்கன லுதவியில்லா வுதரத்தில் வாயுமாற்றும்
பொங்கிய இருமல் மாந்தம் போக்கிடு முண்மைதானே

It relieves *thositha thodam, uthiravaayu*, cough indigestion

வெங்காராஞ் சேத்துமத்தை வேறுபண்ணு மேகடுகு
தங்குசில நீர் முறியத் தான் வாங்கும்

It relieves *kabha* diseases, urinary disorders. It also used in delayed labour, oral ulcer, ulcer in nipple, anaemia due to menorrhagia, dysmenorrhoea, convulsion, post-partum bleeding. It used in delayed labour for uterine contractibility

- Gunapadam thathu jeeva vaguppupage no.326,327.

Externally it is used for antiseptic. It also cures oral ulcer, herpes zoster, renal stone, irritation in urethra, pre-eclampsia. It helps uterine contraction.

- Tamil English agarathi pg no 3896

PURIFCATION OF BORAX:

1. Borax is bundled and hanged in the buffalo's dung solution and boiled. The bundle is cleaned with fresh water and insolates to get it in purified form.
2. Borax is bundled and kept buried in buffalo's dung for 3 days and then washed and dried.
3. It is washed in cow's dung solution.
4. It is soaked in buffalo's urine for 72 minutes (*3Nazhigai).*
5. It is fried in an earthen pan and triturated with vinegar (or) lime juice and dried.
6. It is fried till the moisture completely evaporates.
7. It is triturated with lemon juice or rice washed water and dried.
8. It is soaked in lemon juice or vinegar or five leaved chaste tree leaf juice *(Nocchi* leaf) and dried.

-Gunapadam Thathu Jeeva Vagupu, Page-328

MEDICINAL USES:

- Borax (35 gm) dissolved in water (10.4 Litre) is used as a mouth wash in case of oral ulcers and sore throat. It is also used as a wash for anal fissures and ulcer.
- Borax (4.2 gm) mixed with pig's ghee (21 ml) is applied over the painful anus for anorectal diseases.
- Borax (16.8 gm) mixed with water may be used as an irritation solution for bladder wash in case of urethritis. Borax powder (2.1 gm) mixed with cinnamon bark powder (650 mg) can be given along with gruel 1 to 2 hours four times a day for relief of labour pain during delayed labour. In addition, borax powder (650 mg) is given once in every six hours for pain relief.

- Roasted borax (650 mg – 1300 mg) is given with tender coconut water for urinary tract infection.
- Borax 65 mg to 325 mg mixed with breast milk is given to children for relief of pain and convulsions.
- It is capable of removing stones from the bladder and kidney.
- It can be used during delivery time to increase the labourpains and for fits,
- It cures toad skin, ringworm, peptic ulcer, bladder stone,dysuria etc,
- Its solution is used as lotion and for gargling.
- Take purified borax (4.2gm) and added with purified honey (35gm) and apply for mouth ulcer (stomatitis). It is called as *venkara mathu*.
- Purified borax mix with ghee or butter and used for mouth ulcer, tongue ulcer, fissure in lips. it is refrigent to the body.
- Borax (35gm) dissolved in water (10.4litres) is used as mouth wash in case of oral cancer, sore throat, oral inflammation due to mercury poisoning. It used as gaggling.
- It is also used as external wash for anal fissure and ulcer
- Borax (4.2gm) mixed with water and used as in burning micturition.
- Externally it is used in skin disease, mosquito bite, pricking heat.
- Borax (4.2gm) mixed with vinegar (4.2ml) and externally used for ringworm, venereal itching.
- Borax (260-520 mg) with betel leaf used as fever with rigor.
- Borax (325mg), pepper (195mg), honey (4ml) mixed well and taken for cough, asthma three times per day.
- *Venkaram* used in drug toxicity

  i) *Abrus precatorius* (*Kundrimani*) poison: 2gm purified borax mixed with water and given for abrus poison

  ii) (*Naabi) Aconitum ferox* poison: Purified borax mixed with cows ghee and given for *naabi* poison

  -Agathiyar vidapradhi vida thiratu

OTHER PREPARATIONS OF *VENKARAM:*

1.VENKARA PARPAM:

Dose : 260 – 390 mg (2 – 3 *Kundri)*

Adjuvant : Honey or ghee

Indications : kidney stone, obstruction of urethra, burning micturition,

- Anuboga vaidhiya navaneetham part III, page 24

2.VENKARA CHENDOORAM:

Dose : 650- 1040 mg (5 – 8 *Kundri*)

Adjuvant : Tender coconut water

Indications : *Kalladaippu,* Oliguria

Burning micturition

- Anuboga vaidhiya navaneetham part III, page 27

3.*SIRUNEER KALLUKU KUDINEER*

Dose : 30 – 60ml (0.25-0.5alakku)

Indications : *kalladaippu*,

- Anuboga vaidhiya navaneetham part III, page 24

4.*ARATHARA PARPAM*

Dose : 130mg

Adjuvant : tender coconut water, *venkaaya chaaru*.

Indications : *kalladaippu, sathaiadaippu, neer erichal, neer kaduppu.*

- Anuboga vaidhiya navaneetham part II, page 76

5.ASTA GUNMA THIRAVAGAM

Dose : 5-10 drops

Adjuvant : *chukku kudineer, nerunji vaer kudineer*

Indications : *kalladaippu, neeradaippu, sathaiadaippu, gunmam*

- Anuboga vaidhiya navaneetham part III, page 83

6.*VEDIUPPU SEYANNER*

Dose : 4-10 drops

Adjuvant : *Ilaneer, nerunji vaer kudineer* and fruit juice.

Indications : *kalladaippu, sathaiadaippu, neerkattu and neer erichal.*

- Anuboga vaidhiya navaneetham part III, page 78

7.KAARA SUDA SATHU PARPAM

Dose : 12 – 18 gm

Adjuvant : honey

Indications : *kalladaippu, neeradaippu,*

- Sighicha rathina deepam part II, page 218

8.SATHURMUGA PARPAM:

Dose : 130-260mg

Adjuvant : honey and ghee

Indications : *kalladaippu, neeradaippu, sathaiadaippu*

- Pathartha guna vilakam thaathu jeevam, kannusaamy pillai, page 212

9.ERUKAARA PARPAM:

Dose : 130-260mg

Adjuvant : tender coconut

Indications : *kalladaippu, neeradaippu, sathaiadaippu*

-Pathartha Guna Vilakkam (Thathu Jeeva Varkam) Page No. 174

10.SILASATHU PARPAM

Dose : 130-260 mg

Adjuvant : butter

Indications : *kalladaippu, sathaiadaippu, neeradaippu, neerkadupu and neer erichal.*

- Koshaiye anuboga vaidhya brahma ragasiyam page 110.

3.1.2 GEOCHEMICAL ASPECT:

Borax, a hydrated sodium tetraborate, is one of the most important of boran minerals. Borax is a white to greyish, greenish or bluish coloured granular mineral crystallizing in mono clinic system. It occurs in the form of large transparent prismatic crystals resembling in shape the crystals of augite; also in lumps and compact glassy masses.

CHEMICAL NAME:

Borax, Sodium biborate, Sodium tetraborate

CHEMICAL FORMULA:

$Na_2B_4O_7.10H_2O$

MINIING AND PREPARATION:

Borates are obtained commercially from:

1. Bedded deposits beneath old play as shallow saline and alkaline tertiary lakes.
2. Brines of saline lakes and marshes.
3. Encrustations around playas and
4. Hot springs and fumaroles.

The bedded borate deposits are extracted by underground mining methods and the mined material is crushed and roasted to remove the water separated from the clay and refined to borax. Brines containing borax are pumped out and the various constituents are separated by complicated chemical treatment, which is essentially evaporation followed by fractional crystallization with careful control of temperature and concentration.

During evaporation, the sodium carbonate, sulphide and chloride are precipitated then, when saturation with potassium chloride occurs, rapid cooling causes it to be precipitated and further cooling gives borax and other salts, which are then refined to pure borax. Borax is obtained by boiling native calcium borate with solution of sodium carbonate.

$Ca_2B_6O_{11} + 2Na_2C0_3 \longrightarrow Na_2B_40_7 + 2NaB0_2 + 2CaC0_3$

CalciumBorate + SodiumCarbonate $\longrightarrow$ Borax

CLASSIFICATION OF BORAX

There are 2 types of borax available

- Dana class
- Sturz class

OTHER FORMS OF BORAX:

Borax exists in three forms.

1. **Ordinary or Prismatic borax** which is decahydrate or monoclinic, $Na_2B_4O_7.10H_2O$. This is the common form of borax and is obtained when a solution of the salt is crystallised at room temperature (ie, $< 60^0$).
2. **Octahedral or jeweller's borax** which is penta – hydrate, $Na_2B_4O_7.5H_2O$ and is obtained when the solution is crystallised above 60^0C.
3. **Borax glass or Anhydrous** which is the anhydrous form, $Na_2B_4O_7$ and is obtained by heating the ordinary borax above its melting point until all the water of crystallization is given off. It is a colourless glassy mass (density = 2.37), absorbs moisture from air and gradually converted into decahydrate form.

PHYSICAL PROPERTIES:

Boron trioxide-		36.6
Soda	-	16.2
Water	-	47.2
H	-	2-2.5
Nature	-	Crystalline Lumps
Colour	-	White, sometimes greyish
Streak	-	White, Translucent to opaque
Cleavage	-	Poor
Fracture	-	Conchoidal
Lustre	-	Vitreous to resinous sometimes earthy
Tenacity	-	Brittle
Transparency	-	Translucent
Hardness	-	2 to 2.5
Sp. Gr	-	1.65 to 1.7
Taste	-	Saline alkaline
Mol weight	-	381.37 gm
Density	-	1.71
Habit	-	transparent, prismatic crystals, lumbs glossy masses
Radioactivity	-	Grapi = 0, Borax is not radio active
Solubility	-	Soluble in cold water, Glycerine Insoluble in alcohol

- Internet: wwwmindat.org.

Fatal dose - 15 to 20 grams for adults

5 Grams for children

50gm/100ml blood indicates borax poisoning

-Modis. Medical jurisprudence and toxicology

Borax is sold in transparent, colourless, crystalline masses with coolsaltish odour and styptic taste.

- Tamil - English Dictionary (Tamil *Perakarathy*)

CHEMICAL PROPERTIES:

EFFECT OF HEAT:

1. Heated on a burner flame using blowpipe, bubbles up and fuses to a clear glassy bead.
2. It colours the flame yellow due to sodium and when moistened with sulphuric acid and alcohol, given a green flame due to boron.

$Na_2B_40_7.H20 \rightarrow$ on heating $-10H_20 \rightarrow Na_2B_40_7 \rightarrow B_20_3 + 2NaB0_2$

Boric anhydride

Sodium meta borate

(Glassy Mass)

REACTION WITH ACIDS:

1. With hydrochloric acid gives yellow colour solution in cold condition and on boiling dissolves completely.
2. With sulphuric acid gives colourless solution in cold condition and on boiling dissolves completely.

SOLUBILITY IN WATER:

Borax is completely soluble in purified water producing an alkaline solution as tested by a red litmus paper turning blue.

TEST FOR IDENTITY:

1. A mixture of ethyl alcohol with boric acid burns with a green edged flame due to the formation of ethyl borate.
2. Solution in water in acidic in nature.

TEST FOR PURITY:

It is tested for arsenic, heavy metals, and sulphate and alcohol insoluble substances (determined by dissolving 1 gm in 10ml of boiling alcohol, when the solution should not be more than faintly turbid).

3.1.3. LATERAL RESEARCH

1. MINIMUM INHIBITORY AND MINIMUM BACTERICIDAL CONCENTRACTIONS OF BORON COMPOUNDS AGAINST SEVERAL BACTERIAL STRAINS:

Tuek J Med Sci 2012; 42 (sup.2): 1423 – 1429

ABSTRACT:

Borax compounds are essential micronutrients for many organisms. However, they negatively affect plant, soil, and water micro flora if excessive amounts exist in irrigation water. Therefore, this study aimed to define the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of boric acid and borax by selecting the bacteria that can survive in all environments.

The antibacterial efficacy of boric acid borax against several bacterial strains was evaluated 3 times with the macro dilution broth method.

The MICs and MBCs of boric acid were obtained as 3.80 mg/ml, 3.80 mg/ml, 7.60 mg/ml, and 7.60 mg/ml, against the bacterial activities of Staphylococcus aureus, Acinetobacter septicus, Escherichia coli, and Pseudomonas aeruginosa, respectively. The MICs and MBCs of borax were obtained as 23.80 mg/ml, 23.80 mg/ml, 47.60 mg/ml, and 47.60mg/ml against the above bacteria, respectively.

-Available on: www.dergipark.ulakbim.gov.tr

2. EFFECT OF BORAX ON IMMUNE CELL PROLIFERATION AND SISTER CHROMATID EXCHANGE IN HUMAN CHROMOSOMES

Journal of Occupational Medicine and Toxicology 2009, doi: 10.1186/1745-6673-4-27 Accepted: 30 October 2009. Author Malinee Pongsavee at al.

ABSTRACT

Background: Borax is used as a food additive. It becomes toxic when accumulated in the body. It causes vomiting, fatigue and renal failure. Methods: The heparinized blood samples from 40 healthy men were studied for the impact of borax toxicity on immune cell proliferation (lymphocyte proliferation) and sister chromatid exchange in human chromosomes. The MTT assay and Sister Chromatid Exchange (SCE) technic were used in this experiment with the borax concentrations of 0.1, 0.15, 0.2, 0.3 and 0.6 mg/ml. Results: It showed that the immune cell proliferation (lymphocyte proliferation) was decreased when the concentrations of borax increased.

The borax concentration of 0.6 mg/ml had the most effectiveness to the lymphocyte proliferation and had the highest cytotoxicity index (CI). The borax concentrations of 0.15, 0.2, 0.3 and 0.6 mg/ml significantly induced sister chromatid exchange in human chromosomes ($P < 0.05$). Conclusion: Borax had effects on immune cell proliferation (lymphocyte proliferation) and induced sister chromatid exchange in human chromosomes. Toxicity of borax may lead to cellular toxicity and genetic defect in human.

-http://www.occup-med.com/content/4/1/27

3. HISTOLOGICAL EVALUATION OF THE EFFECTS OF BORAX OBTAINED FROM VARIOUS SOURCES IN DIFFERENT RAT ORGANS

Int. J. Morphol., 33(1):255-261, 2015.

SUMMARY: Boron is an essential element for life and intake via different sources into the body. Because effects of boron and compounds on the body has not been studied enough especially in tissue level, we planned this study to evaluate the effects of borax the most in taken form of boron compound on different intra abdominal organs histologically and also clinically. 42 male rats divided into equal 7 groups and different toxicological doses consistent with its LD50 dose (5000 mg/kg/d) were administered by gavage except control and sham groups. In the study, 2 different kinds of borax one of which was produced for research and the other for agriculture but the same formulation were used and their effects were also compared. As a result it was found that borax did not cause any histological changes in kidney, large intestine, liver and stomach in lower doses. But if doses were increased, a slightly inflammatory cell migration was detected without clinical signs in liver and large intestine. However, when a single very high dose of borax was administered, very high edema, inflammatory cell migration and neovascularization was observed and clinically 2 out of 6 rats died within 5 hours. We suggested that very high dose intake of borax may cause sudden death and also during long periods and higher dose intake may pave the way of inflammatory bowel diseases. At the same time, in boron related studies we advise that the kind of boron and also their source should be evaluated carefully and the most suitable compound should be chosen in case of faulty results.

KEY WORDS: Borax; Inflammatory bowel disease; Histopathology; Toxicology.

3.2 MORINGA OLEIFERA

3.2.1 GUNAPADAM ASPECT

Tamil Name:

Murungai

Botanical Name:

Moringa oleifera

Other Names:

Drumstick tree, Horse Radish tree

நளினமென்ற பாலரசு யென்றும் பேரு

நலமான காரிமூலி யென்றும் பேரு

அளின மென்ற வசுனரி யென்றும் பேரு

அதறஞ்சி வட முருங்கை யென்றும் பேரு

மளின மென்ற மாதவம் பரிலானென்றும் பேரு

மணக்கோல மூலியென்று மதற்குப் பேரு

தெளினமென்று திசை முருங்கை யென்றும் பேரு

திருவான காட்டு முருங்கை யதன் பேரே

Paalarasu, Karimooli, Vasunari, Vada murungai, Madhavam, Manakola Mooli

-Pancha Kaviya Nigandu pg 252

Vernacular Names:

Sanskrit	–	*Shobhanjana*
Hindi	–	*Mungna, Sainjna, Shajna*
Bengali	-	*Sajina*
Marathi	–	*Achajhada, Shergi*
Gujarathi	–	*Midhusaragaru*
Telugu	–	*Mulaga*
Tamil	–	*Murungai*
Kannadam	–	*Nugge*
Malayalam	–	*Muringa*
Assam	–	*Saijna*
Orissa	–	*Sajina*
Punjab	–	*sainjna*

Wealth of India page no. 426

HABITAT

Moringa tree is growing in Himalayan forest naturally. It is cultivated in India, Burma and Sri Lanka. *Gunapadam Mooligai Vagupu pg no 771.*

HABIT: Tree

PART USED: Whole plant

TYPES:

Kodi murungai (Indigofera arcuata)

Kaattu murungai (Hydysarum sennoiles)

Thavasu murungai (Justicia iranquevar)

Gunapadam Mooligai Vagupu page.no 771.

ORGANOLEPTIC CHARATERS

Taste

Bitter, astringent, Sweet

Potency

Cold

Transformation

Unripped fruit – sweet

Bark, Root – sour.

Gunapadam Mooligai Vagupu page.no 771.

ACTION

Antispasmodic

Stimulant

Expectorant

Diuretic

Tonic

Emmenagogue

Abortifaecient

Acrid

Vosiceant

Antilithic *-Gunapadam Mooligai Vagupu page.no 771.*

GENERAL PROPERTIES:

முருங்கைவேர்ப் பட்டைக்கு மூடு கபத்தோ
டொருஞ்குறாச் சன்னிகரம் ஓடும் - அருங்கனக
வட்டைப்பொருமுலையாய் வாய்வொடுவி டங்களுமேற்
பட்டைக்குப் போமே பறந்து

Moringa root bark used for *kapha* disease, *tridosham*

Stem bark used for *vatha* disease and few toxins.

Gunapadam Mooligai Vagupu page.no 773

MEDICINAL USES:

- Stem bark juice and *acalypha indica* (*Kuppaimeni*) juice mixed with gingely oil and apply externally for eczema, scabies. Stem bark juice used in metalic parpam

  Gunapadam Mooligai Vagupu page.no 775

- Moringa stem bark boiled with water and taked it as decotion form to arthritis, paralysis, epilepsy, post portem epilepsy.

  Sarabendirar siddha maruthuva sudar page.no 277

- Moringa stem bark with mustard seed make it into paste form and apply externally to arthritis.

  Pathartha guna bothini page.no 56

- Moringa reduces the body heat
- It relieves joint pain
- It acts as diuretic, expectorant
- It acts on nerves by avoiding epilepsy

  Namnaattu keerai vagaikal, Tamil Nadu Agricultural University,page.no 100.

- All parts of the tree are considered medicinal and used in the treatment of rheumatism, ascites, venomous, bites cardiac and circulatory stimulants.

  -Wealth of India Page.no 426

OTHER PREPARATIONS OF MURUNGAIPATTAI*:*

MURUNGAIPATTAI KUDINEER

Dose : 30 – 60 ml

Indications : *kalladaippu, neeradaippu, sathaiadaippu, vatham, soolai*

-Theriyar Kudineer 100, page 37

NETTI THYLAM

Dose : 4 drops

Indications : *kalladaippu, neeradaippu, sathaiadaippu, gunmam*

- Sarabendirar Vaidhya Rathnavali, page 118

BOOTHA KARAPPAN THYLAM

Dose : 2 drops

Adjuvant : Milk

Indications : *neeradaippu, karappan*

Ennai vagadam, page 40

SARVA NOI LINGA CHENDOORAM

Dose :130-195 mg

Adjuvant : honey and ghee

Indications : *kalladaippu, sathaiadaippu,vaatha pitha kapha diseases*

-Anuboga vaidhiya navaneetham part IV, page 52

***MAHABOOPATHI MATHIRAI*:**

Dose : 1 to 2 tablet

Indications : *kalladaippu, sathaiadaippu,vaatha pitha kapha diseases*

-Kaikanda anuboga Vaidhya perunkural, page 174

3.2.2 BOTANICAL ASPECT

SYNONYMS:

Drumstick tree,

Horse radish tree

TAXONOMICAL CLASSIFICATION:

Kingdom	:	Plant Kingdom
Division	:	Angiosperms
Class	:	Dicotyledons
Subclass	:	Disciflorae
Series	:	Sapindales
Family	:	Moringaceae
Genus	:	Moringa

HABITAT:

A small or medium sized about 10 m. high found wild in the sub Himalayan tract, from Chenap eastwards to Sarda and cultivated all over the plains of India.

HABIT: Tree

PART USED: Whole Plant

A BRIEF HISTORY ABOUT MORINGA OLEIFERA

BARK - Corky bark, grey in colour.
Deeply fissured with easily breakable branches

WOOD - Soft.

ROOT - Pungent

LEAVES - Tripinnate

RACHIS - Slender, thickened and articulation at the base.pinnae of pinnules, deciduous, their rhacchides very slender articulated and with a gland at the articulations.

LEAFLETS - 12-20 by 6-10mm. The lateral leaflets elliptic and the terminal ones obovate and slightly larger than the lateral ones 6-9 pairs pale beneath.

PETIOLE - Slender, petiole of the terminal leaflets is 3-6mm is length.

GLANDS - Linear, hairs panicles spreading.

FLOWERS - White in colour large puberulous axillary panicles, honey scented 1 inch in diameter

CALYX - Linear, lanceolate reflexed.

PETALS – Narrowly spathulate veined.

STAMENS - 5 fertile altenating with 5-7 antherless one

OVARY - Oblong, Villous, hairy.

PODS - Reach up to 45cm length .9 ribbred, pendylous.

CULTIVATION

The tree is indigenous to North - West India and is plentiful on recent alluvial land in or near sandy beds of rivers and streams. It is often cultivated in hedges and homeyards. It grows in all types of soils, except stiff clays and thrives best under thetropical insular climate of South India. The tree can be propagated by seeds or from cuttings. Cuttings are preferred. Plants raised from seeds produce fruits of inferior quality

TYPES

JAFFNA - Grown in parts of South India produces fruits 60 - 90 cm. in length.

CHAVAKACHERI - Also a Jaffna type , bears fruits as long as 90 - 120 cm.

CHEM MURUNGAI - Is a type yielding pods with red tips.

The tree is not affected by any serious disease in India. A foot - rot, caused by Diplodia sp., has been observed in Madras. Two caterpillars and a strmborer are known to affect the tree. The hairy caterpillar, Eitplerote mollifera Wlk., causes defoliation, it is controlled by spraying the tree with fish oil - rosin soap or BHC., or by I buring with lighted torch.

PHYTO CHEMISTRY

Analysis of pods gave the following values, moisture, 86 - 97 protein 2.57 % Fat 0.1% carbohydrate 3.7 %, fiber 8 %, & mineral matter 2 %, Calcium 30 mgs. Phosphorus 110 mg, iron 5.3 mg, copper 3.1 mg, iodine 8 mgs, and oxalic acid are present.

The Leaves are rich in carotene and ascorbic acid analysis gave the following values moisture 75 % protein 6.7 % Fat 0.7% carbohydrate 13.4 %, fiber 0.9 %, &mineral matter 2.3 %, Calcium 440 mg, Phosphorus70 mg, iron 7 mg. Copper 1.1 mgs, iodine 5 mg are present .

The flowers contains traces of alkaloids, they also contain acid value 10.5 , the ash is rich in potassium and calcium.

The seeds are oleaginous. Analysis of the kernel gave the following values, moisture 4 % protein 38.4 %, fat 34.7 % , carbohydrate I % , fiber 8.5 % , & mineral matter 3.4 % .

ALKALOIDS

The bark contains two alkaloids moringine which is identical with benzylamine and moringinine belonging to the sympathomimetic group of bases. An alkaloid named spirochin, has been isolated from the roots.

ANTIBIOTICS

Antibiotics - Pressed juice of the leaves of the plant show strong antibacterial activcity against Micrococcus pyogenes var. aureus, Escherichia coli and Bacillus subtilis. The leaf juice is bacteriostatic in a dilution of 1:1,00,000.

The roots contain an active antibiotic principle, pterygospermin which is obtained as a low - melting unstable substance with a characteristic odour, soluble inorganic solvents but benzyl isothiocyanate, it is more stable in phosphate buffer than water. Pterygospermin inhibits the growth of many Gram positive and Gram-negative bacteria. In higher concentrations, it is active against fungi.

The Gums stem of the tree exudes a gum which is initially white in colour butchanges to reddish brown or brownish black on exposure. It is a polyuronide consisting of arabinose, galactose and glucuronicacid .Rhamnose is present in traces.

3.2.3 LATERAL RESEARCH

1. DIURETIC ACTIVITY OF MORINGA OLEIFERA LEAVES EXTRACT IN SWISS ALBINO RATS

The pharma innovation journal (www.thepharmajournal.com) 2016, 5(3): 08-10 ISSN: 2277- 7695 Author : Rohithsingh Tahkur, Geetha Soren at al

ABSTRACT:

Background: Moringa Olifera commonly known as drumstick belongs to the Moringaceae family. A number of medicinal properties attributed to different parts of M. Olifera. we evaluated diuretic activity of alcoholic extract of Moringa oleifera leaves in swiss albino rats compared with hydrochlorothaizide. **Methods:** In first group, six albino rats was kept as control, was given only 0.9% normal saline 25ml/kg body weight orally. Another group of 6 rats were fed with normal saline 25ml/kg along with standard hydrochlorothiazide 2.5mg/kg. The Third, fourth and fifth groups of 6 rats each were taken as test group and the crude extract of Moringa Oleifera which was obtained in liquid form along with normal saline was given, keeping the volume constant, in doses of 50,100 and 200mg/kg bodyweight. Metabolism cage was used to collect urine in beakers for a period of 5 hours and 24 hours. Analysis of the data was done using ANOVA and Tuckey test. P values of less than 0.05 were considered significant. **Results:** After 5 hours of urine analysis-The urinary volume of the control group was 7.3±0.2 mL and the urinary excretion of Na+, K+ and Cl- are 77.7±1.2 mEq/L, 21±1 mEq/L and 262.2±0.5 mEq/L respectively. The urinary volume of the standard group was 13.37±0.95 mL and the urinary excretion of Na+, K+ and Cl- are 168.4±3.39 mEq/L, 16±0.62 mEq/L and 147.46±5.79 mEq/L. After 24 hours of urine analysis-The urinary volume of the control group was 13.7±0.5 mL and the urinary excretion of Na+,K+ and Cl- are 63.72±0.56, 22.05±0.34 and 265.5±1 mEq/L respectively. The urinary volume of the Standard group was 4.13±0.73 mL and the urinary excretion of Na+,K+ and Cl- are 173±45, 20.01±0.15 and 151.41±6.52 mEq/L **Conclusion:** Moringa oleifera leaves extract produced dose dependant diuretic action which is greater than control lesser than hydrochloro thiazide. The extract showed dose dependent saluretic effect. On the other hand, potassium sparing activity was not observed.

Keywords: Moringa Olifera, hydrochlorothiazide, Diuretic Action, Swiss albino Rats, Metabolism cage

2. PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATIONS ON THE BARK OF MORINGA OLEIFERA LAM

Indian Journal of Natural Products and Resources, Vol. 4(1), March 2013, pp. 96-101

Author : Sholapur, Hansan Pasha N.Patil, at al

ABSTRACT

Moringa oleifera Lam. belonging to the Moringaceae family is a highly valued plant, distributed in many countries of the tropics and subtropics. It's some of the common names include Horseradish tree, Drumstick tree, Benzolive tree, Shajna, Nugge mara and Sigru. The bark is widely used as emmenagouge, rubefacient, anticancerous, antitubercular, antifungal, cardiac and circulatory stimulant. It is necessary to ascertain the authenticity of this crude drug when it is used for therapeutic purpose. Hence, the present study was under taken for systematic pharmacognostical evaluation of the bark of the plant with respect to macroscopy, microscopy and physico-chemical parameters. The TLC profile was developed for petroleum ether and ethanolic extract of the bark. Preliminary phytochemical investigation indicated the presence of carbohydrates, triterpenoids, isothiocynate glycosides, tannins and steroids. These established parameters could be used in identification and authentication of the crude drug. Keywords: Moringa oleifera, Horseradish tree, Drumstick tree, Bark, Macroscopy, Microscopy, Fluorescence analysis, Phytochemical.

3.3 UPPILANG KODI

3.3.1 GUNAPADAM ASPECT

Tamil name:

Uppilang kodi

Botanical name:

Pentatropis microphylla

Other names:

Ambarvel, Uppili, Uppili Ver, Parparam

Vernacular Names:

Sanskrit	:	*vlauka, dhidaparni, kaka nasa, kaka nasika, shringarati, suryavalli, sunasika.*
Marathi	:	*shingrota*
Malayalam	:	*parpparam, paparam.*
Hindi	:	*ambervel.*
Telugu	:	*chekurtitivva, pulapala*

PART USED:

Leaf, stem, root

ORGANOLEPTIC CHARACTER

Taste	:	slightly sour
Potency	:	heat
Transformation	:	sour.

ACTION:

Digestive,

Febrifuge,

GENERAL CHARACTER:

பிள்ளையின் மந்தபேதி பேராத சூட்சையும் போம்
வெள்ளைசெயுந் தங்கத்தை மென்மலராற் - றோள்ளைபுரி
வேளு நடுங்குவடி வேலைநிகர் கூர்விழியாய்
நாளுமுப்பி லாங்கொடியி னால்

It cures indigestion for child, milky diarhoea and uncured fever.

MEDICINAL USES:

- It cures stomach bloating due to indigestion when the stem tied over hip region.

- Leaf juice mixed with breast milk given for indigestional diarhoea.
- Leaf used for silver and gold parpam preparation.

Gunapadam Mooligai Vaguppu Page No

- Taking warm leaf juice as nasal drops to alleviate headache, running nose and bodyache.
- Leaves of this plant are boiled with coconut oil and externally used in cuts and wounds.

Indian Materia Medica Page No:652

OTHERS PREPARATION OF UPPILANGKODI

CHANDAMARUTHA KARUKU

Dose : 130 mg

Adjuvant : honey

Indication : stomach pain, diarhoea

-Agathiyar Vaidhya Kaviyam 1500 pg 654

RAGURAMA BAANAM (THUTHA CHENDOORAM)

Dose : 100 mg

Adjuvant : honey, hot water

Indication : Bloating of stomach, *Seetha kalichal,*

-Agathiyar Paripooranam 400 pg 682

KAALA SARAGATHI KASHAYAM

Dose : 30 – 60 ML

Indication : *Soolai,* vomiting, indigestion

-Agathiyar 2000 Part-3 pg 469

UPPHILI NEI

Dose : 10-15 ml

Adjuvant : hot water

Indication : Bloating of stomach, stomach pain

-Pillai Pinni Maruthuvam pg 382

3.3.2 BOTANICAL ASPECT

TAXONOMICAL CLASSIFICATION

Kingdom	:	Plant Kingdom
Division	:	Angiosperms
Class	:	Dicotyledons
Subclass	:	bicarpellatae
Series	:	gentianales
Family	:	asclepediaceae
Genus	:	pentatropis.

HABITAT:

In tamilnadu, it is growing in guardian region, mountain region. Leaves will be thickened but broken easily. It occurs in semi-arid thorny forest and thick forest.

HABIT:

It is a twinning perennial herb on shrubs and trees

A BRIEF HISTORY ABOUT PENTATROPIS MICROPHYLLA

Leaf	:	1 to 3.5 cm long 0.5 to 2.5 cm wide, broadly oblong or ovate, elliptic, tip is blunt with a short point, base rounded. Lamina is chartaceous Leaf stalk is 4-7 mm long, Milky latex present in leaf.
Inflorescence:		Axillary ambel type
Flowers	**:**	Flowers are pentamerous. Flowers clusters are carried on a short stalk upto 2mm long. Flowers stalk are 1.2 to 1.3 m long
Sepals	:	Sepals are 1.5mm long. Five greenish sepals, Flowers tube is almost divided to the base
Petals	:	3.5 to 6mm long.Five lobes of purplish corolla. corona lobes are 2 -2.5cm long
Follicles	:	3.7 to 6.8cm long
Seeds	:	6×3 mm. ovate, tip truncate slightly crenulate at the base.

MEDICINAL USES:

- The plant is considered as cooling and alternative agent.
- The plant is yields a bio active compound cardiac glycoside. It is used to treat constipation, colic and diarhoea.
- In siddha system it is used as paediatric medicine especially children suffering from digestive problem and severe fever.
- The plant extract also contain anti-astringent principle
- In ayurveda it is equivated as kaka nasika useful in upper respiratory infection.

PHYTO CHEMICALS

- Chemically the source taxon is rich in compounds such as octacosanal, α amyrin, friedelin, β-sitosterol and sallicyclic acid.

3.3.3 LATERAL RESEARCH

1. PHYTO CHEMICAL, ANTI FUNGAL, ANTI MICROBIAL AND ANTI OXIDANT STUDIES ON WHOLE PLANT EXTRACT OF PENTATROPIS MICROPHYLLA

Available online at www.ijpcbs.com IJPCBS (International Journal of Pharmaceutical, Chemical and Biological Sciences) Mohan Gandhi et al. 2012, 2(4), 453-463 ISSN: 2249-9504 453

Author : Mohan Ganthi Bonthu at al

Abstract

The genus pentatropis is considered as one of the most important genus used in various systems of medicine due to their reported variety of compounds. In the present investigation the plant, Pentatropis capensis, was examined chemically for its phyto constituents. Investigation revealed the positive results for the presence of steroids, flavanoids, tannins and glycosides. The chloroform extract of stem showed moderate activity against bacterial organisms like Staphylococcus aureus, Bacillus subtilis, and Escherichia coli. The chloroform extract of the whole plant showed no antifungal activity at concentrations of 100mg/ml, 300mg/ml against fungal organisms. Methanollic extract of the plant showed significant effect on hydrogen peroxide radical scavenging activity and less significant effect on nitric oxide radical scavenging activity when compared with the standard.

Keywords: Pentatropis capensis, Scavenging activity, Gram + Ve, Gram –Ve

2. AN OVERVIEW OF SOME PROMISING MEDICINAL PLANTS WITH IN VITRO ANTI-UROLITHIATIC ACTIVITY

IOSR Journal of Pharmacy (e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219 (www.iosrphr.org) Volume 5, Issue 5 (May 2015), PP. 23-28 23 Author : Anand Tiwari, Vivek soni at al

Abstract:

Kidney stone formation is so acute in some places that they are called stone belts and Gujarat is one of them. Though most prevalent and widespread disease in the world no guaranteed cure is found till date. None of the known and available treatments prevent the reoccurrence of kidney stone formation. Hence a dire need for herbal formulation appears to be the need of the hour. This present review discuses

the causes, cure and treatment of kidney stone formation. It emphasizes herbal remedies and lists of some of the promising plants which show in vitro anti urolithiatic activity. They can be further taken up for in vivo studies or may be used in herbal formulations and find a new herbal drug to treat the dreadful diseases

Key words: kidney stone, anti urolithiatic activity, in vitro, medicinal plants, herbal formulation.

3. ANTIMICROBIAL ACTIVITY OF PENTATROPIS MICROPHYLLA LEAVES

International Journal of Pharm Tech Research CODEN (USA): IJPRIF ISSN: 0974-4304 Vol.2, No.3, pp 2022-2024, July-Sept 2010 Author : M.Ramaprabha and K.Vasantha

Abstract:

Phytochemical screening and antimicrobial activity of acetone and methanolic extracts of Pentatropis microphylla L. (Asclepiadaceae) leaves against various pathogens such as Salmonella paratyphi, S. paratyphi A, Bacillus subtilis, B. thuringiensis, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and fungal pathogens like Fusarium oxysporum, Aspergillus flavus, Mucor sp., Cladosporium sp. were analysed in the present study. Phytochemical screening recorded positive results for alkaloids, tannins, steroids, glycosides, saponins and flavonoids. Among which flavonoids, phenols and steroids showed maximum degree of precipitation. Negative results are obtained for fixed oils. Maximum antibacterial activity was showed against Pseudomonas aeruginosa and Salmonella paratyphi B.

Key Words: Pentatropis microphylla, phytochemical, antimicrobial.

3.4. REVIEW OF DISEASE

KALLADAIPPU

3.4.1 SIDDHA ASPECT

Siddhars classified diseases into 4448 types. One among them is "*Kalladaippu Noi*".The most prominent and ancient siddha literature is*"Yugivaithiya chinthamani 800".*

In Siddha system, according to *Theran Karisal,* Urinary disease are classified into

1. *Neerinai perukkal Noi*
2. *Neerinai Arukkal Noi*

நீரிருவினைக் குணத்தை நீயறிவித்துச் சொல்வோம்
நீரினைப் பெருக்கலொன்றே நீரினை யருக்க லொன்றே
நீரிழிவுடனே கொல்லும் நீர்க்கட்டு வினைகளொன்று

-தேரன் கரிசல்

Kalladaippu is one of the urinary disease which comes under Neerinai *Arukkal Noikal.*

1. **SYNONYMS**

Aachamari

2. DEFINITION

A) Sudden Obstruction to the flow of urine, pain in the base of penis in males and clitoris in females, burning sensation in external meatus, joint pain, passing of small sand like stones along with urine is the cardinal features of this disease.

-Siddha maruthuvam

B) Indicates a few features of *Kalladaippu* in the following poem

விழுகுஞ்சில நேரம் விடுபட்டு நீரோடும்
ஒழுகிய வாயவ மொதுங்கினால் நோகாது
வழுகிய மந்தத்தால் வாயுவந்தே புகில்
கழுகி முதிர்ந்திடும் கல்லடைப்பாகுமே.

-Thirumoolar karukkandai vaidhyam

C) தானென்ற முத்திரத்தில் நறநறவென்று
தங்கியதோர் பொடியெனும் மணல்தானமப்பா
வானென்ற சிறயதொரு கல்லாவதப்பா
வளமாக வந்து விழும் நோய்க்குத் தானே
ஏனென்ற அஸ்மரி ரோகமென்ற பேராம்

According to the above quote, *kalladaippu noi* is due to "deposition of sand-like grains in urine, which results in formation of gravel, followed by expulsion of stones during micturition". Further, he elaborates that the stones cause obstruction in the urinary tract including the kidneys. As the stone surpasses the urinary tract, excruciating pain begins and intensifies. When the stones reached the uretheral orifice extreme burning sensation occurs in the external genitalia and the pain subsides instantly once when the stone gets expelled.

-Agasthiar gunavagadam

3. ETIOLOGY FOR RENAL CALCULI:

(i) In Siddha system many causes of this disease are mentioned.

அறைகிறேன் விந்தழிந்தால் மேகமாச்சு
அமுது வழிந்து சுடுகொண்டால் வாயு சேரும்
பறைகிறேன் சுலை குட்டம் கிரந்திப் புற்று
பவுத்திர கள் பிளவை போட்டு கண்டமாலை

அறைகிறேன் அரையாப்பு ஒட்டிய புஜ
அருங்கரப்பான் சிரங்கு குன்மம் மாநீர்க்கட்டு
குறையாவே நீர் கொண்ட அந்நோய் காசம்
குடிலமாம் பேதியோடு கிராணி பாண்டே

The above poem describe that, when the semen is destroyed by body heat. *Vatham* will add with that, and many diseases will come, including *kalladaippu.*

-Agathiyar vaithiya vallathy600.

(ii) கலங்கினதோர் தண்ணீர்கள் குடித்த போக்குங்
கல்லெலும்பு மயிர் மண் தான் கலந்தன்னத்தில்
அலங்கினதோ ரன்னங்களருந்தலாலும்
மலங்கினதோர் மாபண்ட மருந்தாலும்
மந்தத்தில் வாயவான பதார்ததந்தன்னை
துலங்கினதோர் ருசிதன்னதிற் சுவைத்தலாலும்
சுருக்காய்க் கல்லடைப்பு வந்து தோன்றுந்தானே.[40]

The above poem describes that chronic *mega noi* produces small stones in the bladder and vitiation of *vatham* and *pitham* these small stones become larger in size and block the urinary passage.

Urinary stones are also formed due to the drinking of contaminated water, taking of food mixed with sand small stones, consuming of contaminated food articies, food containing more carbohydrates, unhealthy food habits, etc.

-yugi vaidhya chinthaamani

(iii) நீரினைத் தடுத்தல் செய்யின் நீர்கட்டுத் துவாரம்
பாறிடுஞ் சந்து சந்தில் பண்புறு நோவ தாகும்
நேரிலங் கயருங் காம்யம் நிச்சய நோதல் செய்யும்
பூரினி லபான வாயு பண்புறச் சேருமன்றே
சுக்கலந் தனைய டக்கின் சுரமுட னீர்க்கட் டாகும்
பக்கமாங் கைகால் சந்து பாரநோய் வழியிற ங்கும்
மிக்கமோர் நோயுண் டாகும் மிகுத்திடும் பிரமே கந்தான்
தக்கதோர் போதுமாகின் தரித்திடும் வாயுக்குறே.

Urine and semen are the natural urges in the body. Suppression of excreation of urine or seminal discharge is one of the predisposing causes for the formation of stone in the bladder.

-Siddha maruththuvanga churukkam

4. COMMON SIGNS AND SYMPTOMS

1. Sudden obstruction to the flow of urine.
2. Scanty urination.
3. Frequency of micturition.
4. Excruciation pain in the penis and anus.
5. Pain will radiate from loin to groin sometimes radiates to thigh.
6. Some stones are very sharp and irregular during its movement along the urine it will produce intolerable abdominal pain.
7. Burning micturation and haematuria.
8. Vomiting.
9. Headache.
10. If the surface of the stones is rough and irregular with horny projections it may cause extreme pain in the lower abdomen, burning sensation in the urethral orifice and even haematuria.

5. *TYPES OF KALLADAIPPU*

In Siddha System, the *Kalladaippu* is classified according to its sign and symptoms. Types of *Kalladaippu* in some Tamil Siddha texts are dealt with here.

A) ACCORDING TO *YUGI VAIDHYA CHINTHAAMANI*

தோன்றிடதோர் நாலினிட நாமங்கேளாய்
சுறுக்கான வாதத்தின் கல்லடைப்பு
பூன்றியதோர் பித்தத்தின் கல்லடைப்பு
தீன்றியதேர் தொந்நமாங் கல்லடைப்பு
தேகத்தைப் பற்றியே சிறிது காலம்
தான்றியே சலப்பையில் வந்திழிந்து
கருவியே லிங்கத்திற்குந் றரிக்குந் தானே

Four types, these are

ValiKalladaippu, Azhal Kalladaippu, Iyya Kalladaippu,Mukkutra Kalladaippu

B) ACCORDING TO *SIDDHA ARUVAI MARUTHUVAM*

Four types, these are

Vali Kalladaippu,
Azhal Kalladaippu,
Iyya Kalladaippu,
Venneer Kalladaippu

C) ACCORDING TO *THANVANTHRI VAITHIYAM*

Four types of *ashmari,these are*

Kallarippan,
Pitha ashmari,
Slathma ashmari,
Sukkila ashmari

6. SIGNS AND SYMPTOMS

A) ACCORDING TO *NOIVILAKKAM,*

1.VALI KALLADAIPPU

படர்மிகப் படுதல் பற்கள் கடித்தல்
நடுங்கல் உந்தியுங் குறியும் பிசைதல்
கசடுகீழ் வளியொரு கழலல் அழுதல்
சிறுநீர் துளித்தல் என்னாவும் பிறவும்
வளிபின் கல்லடைக் குறியென மொழியே.

Tongue biting, palpitation, shivering, crushing pain of the lower abdomen and genital organ, dribbling of urine, the stone are blackish red in colour.

2.ANAL KALLADAIPPU

சுட்டென நிரியம் மிகவெம் பிடுதலும்
நோமலும் அலைக் கல்லடைக் குறியே
சிவந்துங் கறுத்து மஞ்சனாகியும்
சேங்குரு வடிவில் கல்லது தோன்றும்.

Burning micturition,dysuria the stone are reddish black (or) yellow in colour and passing small stones.

3.IYYA KALLADAIPPU

நீரியங் குத்தல் திணித்தல் குளிர்தல்
எனுமிவை ஐயக் கல்லடைக் குறியே
வெளுத்தும் தேனிற மாகியு மொளிர்ந்தும்
பெருவடி வுடைத்தாம் ஐயக் கல்லடை.

Pricking pain, forceful pain with severe intensity when passing urine, fever with rigor, white (or) honey coloured, shining (or) laminate larger size stone expelled.

4.KARUNEER KALLADAIPPU

கருநீரட்க கலின் வளி சினந்தெளுந்து
விரைனளி னடுவில் அதுதனை தடுத்தலின்
கருநீர் கல்லடை மருவிடு.

Burning micturition, dysuria, stone black in colour,larger size stone expelled

B) ACCORDING TO *YUGI VAIDHYA CHINTHAAMANI*

1.VALI KALLADAIPPU

Acute pain in lower abdomen, swelling in abdomen, urinary flow is uncontinuous so pain present in penis, sometime mucous discharge in urine, patient unable to sit.

2.AZHAL KALLADAIPPU

Obstruction of urinary flow, burning sensation, acute pain in urethra, excretion of small red coloured stones

3.IYYA KALLADAIPPU

Severe pain present in umbilicus, pain radiating towards the thigh, burning micturation, excess sweating and small white colour stone sometimes comes with urine.

4.MUKKUTRA KALLADAIPPU

It is also called *Venneer Kalladaippu* (or) *Manarkalladaippu.*

Pain in tip of urethra, irregular urinary output and small stone come along with urine and semen.

7. PATHOGENESIS

The three *Uyir Thaathukkal* are formed by combination of the three important *Naadi* with *three Vaayus* as follows,

Idakalai + Abaanan = Vatham

Pinkalai + Praanan = Pitham

Sulumunai + Samaanan = Kapam

The *Thiri Thaathukkal* tend to be in their states of equilibrium (1: ½: ¼) during normal conditions of human beings.

ஊற்றதோர் உடலின் கூறு
உறுப்புடன் விரவி நின்று
முற்றுமே நோய்கள் எல்லாம்
முதலதனிலே தோன்றும் போது
பற்றுமே வாத பித்த
சிலேற்பணற்தனந்தன்னில் ஒன்றைப்
பற்றியே தோனற்றுமென்று
பகர்த்தனர் முனிவர் தாமே.

-அகத்தியர் குருநாடி

According to the basic principles of siddha a disease and its manifestations are a result of the derangement in the above said *mukkuttrams*, the result of disturbed *kutrams (ie. vatham, pitham, kapam)*

Through the above poem quoted in *siddha maruthuvanga surukkam*. siddhars emphasize that any changes in diet, water intake and lifestyle leads to a hike in the *vatha* and *pitha kutram* in the body, causing stagnation of urine in the kidneys. If abanan is favourable in the body then these deposits will automatically be expelled through urine. if not, they remains in the remain in the urinary tract leading to *kalladaippu noi*. Whatever may be the causes attributed to the occurrence of *Kalladaippu* or any other disease, the siddha concept is that the manifestation of disease is the result of disturbed *doshas* i.e. *Vatham, Pitham & Kabam*.

வாயுவினாலே மலசலங் கட்டிடும்
பிரிந்திடும் பித்தம் பேராஞ் சலத்தினிலே.

-சித்த மருத்துவாங்கச் சுருக்கம்.

In *kalladaippu*, due to alteration in once diet and water intake,*Vatha* and *Pitha dosham* increase in the body and salts in the urine get sedimented in the kidney. If *abaana vayu* is favourable these sedimented deposits get excreated in the urine and if the *Abaana Vayu* is unfavourable the urinary sediments do not get excreated and get deposited in at anywhere in the urinary pathway and creates this disease.

3.4.2 MODERN ASPECT

RENAL CALCULI (UROLITHIASIS)

1. DEFINITION

A Kidney is a hard, crystalline mineral formed within the kidney or urinary tract. Kidney stones are a common cause of blood in the urine (haematuria) and often severe pain in abdomen flank, or groin, Kidney stones are sometimes referred to as renal calculi.

2. PREDISPOSING FACTORS FOR KIDNEY STONES:

Environmental and dietary causes:

a) Low urine volumes : high ambient temperatures, low fluid intake
b) Diet : high protein, high sodium, low calcium
c) High sodium excretion
d) High oxalate excretion
e) High urate excretion
f) Low citrate excretion

Acquired causes:

a) Hypercalcaemia of any cause
b) ileal disease or resection (Increases oxalate absorption and urinary excretion)
c) Renal tubular acidosis type 1 (distal)

Congenital and inherited causes:

a) Familial hypercalciuria
b) Medullary sponge kidney
c) Cystinuria
d) Renal tubular acidosis type 1 (distal)
e) Primary hyperoxaluria

3. TYPES OF URINARY CALCULI

There are four main types of urinary calculi. They are,

a) Calcium containing stones
b) Mixed (struvite) stones
c) Uric acid stones
d) Cystine stones

In addition to these four types, there are few rare types, occurrence of which is less than 2%.

a) **CALCIUM STONES**

Calcium stones are the most common comprising about 75% of all urinary calculi. They may be pure stones of calcium oxalate (50%) or calcium phosphate (5%) or mixture of calcium oxalate and calcium phosphate (45%).

a) MIXED (STRUVITE) STONES

15% of urinary calculi are made of magnesium-ammonium-calcium phosphate, often called struvite, hence mixed stones are also called as 'struvite stones' or triple phosphate stones.

b) URIC ACID STONES

Approximately 6% of urinary calculi are made of uric acid. Uric acid calculi are radiolucent unlike radio-opaque calcium stones.

c) CYSTINE STONES

Cystine stones comprise less than 2% of urinary calculi

d) OTHER CALCULI

Less than 2% of urinary calculi consist of other rare types such as due to inherited abnormality of enzyme metabolism .ex. hereditary xanthinuria developing xanthine stones.

4. SALIENT FEATURES OF URINARY CALCULI

Table no: 1

Type	Incidence	Etiology	Pathogenesis
Calcium stones	75%	Hypercalciuria with or without hypercalcaemia; idiopathic	Supersaturation of ions in urine, alkaline pH of urine; low urinary volume, oxaluria and hyperuricosuria
Mixed stones	15%	Urinary infection with urea-splitting organisms like proteus	Alkaline urinary pH Produced by ammonia from splitting of urea by bacterially produced urease

Uric acid stones	6%	Hyperuricosuria with or without hyperuricaemia (e.g. in primary and secondary gout)	Acidic urine($_p$H below 6) decreases the solubility of uric acid in urine and favours its precipitation
Cystine stones	2%	Genetically-determined defect in cysteine transport	Cystinuria containing least soluble cysteine precipitates as cysteine crystals
Other types	<2%	Inherited abnormalities of amino acid metabolism	Xanthinuria

5. MORPHOLOGY

CALCIUM STONES

1. Usually small, ovoid (less than a cm)
2. Hard with granular rough surface
3. Dark brown due to old blood pigment deposited in them as a result of repeated trauma caused in the urinary tract by these sharp edged stones.

MIXED (STRUVITE STONES)

1. Yellow-white or grey
2. Soft and friable and irregular in shape
3. Stag-horn stone which is a larger, solitary stone that takes the shape of renal pelvis.

URIC ACID STONES

Smooth, yellowish-brown, hard and often multiple

CYSTINE STONES

Small, rounded, smooth and often multiple

Yellowish and waxy

6. SIGNS AND SYMPTONS

1. In the kidney fixed renal pain, (flank pain)is common.
2. In the ureter (according to Localisation of stone)
3. If in upper 1/3 of the ureter pain radiates to the perineum if the pelvic brim- pain radiates to the inner aspect of thigh.
4. If present in middle 1/3 of ureter –pain radiates to the iliac fossa.

5. If the stone is localised in the bladder neck (or) Urethra.
6. Pain may presence as tip of penis.
7. Frequently of Urination.
8. Oliguria.
9. Dribbling of Urine.
10. Hematuria.

7. PATHOGENESIS OF STONES

1. The mechanism of Calcium stone formation is explained on the basis of super saturation of ions of forming the stone and the concentration of inhibitors in the urine most likely site where the crystal of Calcium Oxalate and /or Calcium Phosphate are precipitated is the tubular lining (or) around some fragment of debris in the tubule acting as nidus of the stone. The stone grows, as more and more crystals are deposited around the nidus. A number of other predisposing factors contributing to formation of Calcium stones are alkaline Urinary PH decreased Urinary Volume and increased excretion of oxalate and uric acid.
2. The solubility of uric acid at PH of 7 is 200mg/dl while at PH of 5 is l5mg/dl. Thus as the urine becomes more acidic, the solubility of uric acid in urine decreases and precipitation of uric acid crystals increases favouring the formation of uric acid stones hyperuricosuria is the most important factor in the production of uric acid stone, while hyperureamia is found in about half the case.
3. Excessive excretions of cystine stones are associated with cystinuria due to genetically determined defect which is least soluble of the naturally-occuring aminoacid leads to formation of crystals and eventually cystine calculi.

8. DIAGNOSIS OF KIDNEY STONES:

Diagnosis of kidney stones requires a complete health history assessment and a physical exam. Other tests include:

1. Blood tests for calcium, phosphorus, uric acid and electrolytes.
2. Blood urea nitrogen (BUN) and creatinine to assess kidney functioning.
3. Urinalysis to check for crystals, bacteria, blood and white cells.
4. Examination of passed stones to determine type.

9. COMPLICATION OF RENAL CALCULUS

1. Azotemia: Increased urea and nitrogen containing compounds in the blood due to inability to excrete the above compounds.
2. Acquired distal renal tubular acidosis
3. Urinary tract infection due to urinary stasis
4. Hyperkalaemia
5. Renal hypertension
6. Chronic unilateral or bilateral hydronephrosis in the presence of ECV Expansion or other renal disease
7. Polycythaemia – infrequent complication of obstructive uropathy secondary to increased erythropoietin production by the obstructed kidney.

3.5 PHARMACEUTICALS REVIEW

CHUNNAM

Definition:

The word 'Chunnam' indicative of an alkaline product similar to lime. In Tamil language, Caustic lime is also turned as Chunnam.

Raw meterials in chunnam preparation:

Many metallic and non metalic mineral drugs are made into chunnam preparations.

Metals: Gold (*Thangam*), Silver (*Velli*), Copper (*Chembu*), Iron (*logam*), Zinc (*Naagam*), lead (karuvangam), etc.

Mercurials and other toxic substance: Mercury (*Rasam*), Cinnabar (*lingam*), Calomel (*Pooram*), Mercuric perchorid (*veeram*), thalagam (As_2S_3)

Salts (karasaram): fuller's earth (*Pooneeru*), salt peter (*vediuppu*), Camphor (*Karpooram*), Kalluppu (salt),etc.

Mineral origin: (*Uparasam*) Mica (*Abrakam*), asbestos (*kalnar*), Copper Sulphate (*thurusu*), Chalcopyrite(nimilai), etc.

Zoological product: Kizhinjal (conch shell), Sangu, nandu odu, nathai odu, Muttai odu.

Equipments required:

- Mortar and pestle
- Vessels and spoons to handle liquids.
- Long ribbons of tough cloth and fine clay.
- Pairs of shallow earthen discs of identical dimensions.
- Cow dung cakes, sufficient numbers and well dried.
- Fine cloth pieces for filtering juices and decoction.
- Spatula for handling powders.
- Air tight containers.

General Method of Preparations:

The above naturally obtained ingredients are processed sequentially by elaborate processes like grinding, melting, triturating, quenching in different plant juices with different heat treatments thus changing form from one to another and in the end, making into bio available form.

Chunnam are prepared by two different methods one is simple and another is complex. *Chunnam* should be prepared during the hot. Summer months like April and May. Simple, Complex method, Crucibles (*moosai*) used in chunnam.

a) Simple Method

In this method either lime stone or calcium group of raw materials are subjcctcd to calcinations or incineration in pudam with cow dung cakes as specified in the formula. Commonly these medicines are used extensively by physicians examples are chunnam as salt peter (vediuppu chunnam) and chunnam of egg shell (anda odu chunnam), which are used extensively in urinary, genital disordcrs and in acid peptic diseases.

b) Complex method

A very systematic, elaborate, stepwisc procedurc is followed in these preparations. High metals, gems or any toxic material is detoxified as per recipe and then converted it to a fine homogenous substance by triturating with plant juices or some distillates called *pugai neer or thiravagam* or with any solvent (jeyaneer) and made into fine paste and are well dried. Tins paste is kept in distinct, specially made crucibles called *moosai* like *Panchachunna gugai* and scaled. After drying, this crucible is heatcd in a hand bellows blower. After sufficient heating, generally up till a point when the crucible becomes red hot and itself gives up its structure. The contents are allowed to cool and collected. The final product after sufficicnt grinding

(i) Crucibles (*Moosai*) Used in Chunnam

It is very interesting to note that heat resistant crucibles (*moosai/gugai*) are specially made prior to chunnam preparation Special crucible called panchachunna gugai is prepared with chunnam of crab shell (nandu), water shell (nathai), conch shell (sangu), Pearl oyster shell (chippi), egg shell (anda oodu). latex of Calotropis erukkampaal. Egg albumin, venkaram and lime water (chunna neer). Anju chunna gugai, pancha bootha gugai are few other crucibles particularly used for chunnam preparation.

(ii) Product Grades:

The chunnam of many metal and non-metal drugs are graded according to the solvents used in the preparation, the type of crucibles used and of heating appliances used in the process of particular preparation. If the chunnam is prepared in pancha chunna gugai and incinerated with bellows blower is graded as best quality. If the

chunnam is prepared with fullers earth (pooneeru chunnam) the drug is graded as second quality. If chunnam is prepared by the use of copper sulphate chunnam it is classified as third quality. If chunnam is prepared with caustic solvents like jayaneer, this variety is classified into 4th quality. Generally physicians prepare a special kind of solvent called chunna thiravagam to prepare all kinds of chunnam of salts, minerals, metals and various gems.

Shelf Life:

When properly stored, they retain then potency up to 500 years.

Adjuvant:

It is advised to take the chunnam medicine with ghee, butter or milk. Dosage is indicated according to the disease condition as 3 days or 7days or 21 days.

Colour:

Chunnams are in powder form and generally soft white to ivory color or colourless.

If white color is not obtained fuller's earth chunnam is added to get the white colour

Character and tests for chunnam:

Chunnams are odourless, lusterless, smokeless on heating.

Microfine in particle size wafer like very light in cut

The end point on purity of chunnam is ascertained by adding a pinch of turmeric with few drops of water it turns turmeric from yellow to red perhaps as on indicater of the pH value.

Chunnam also taste like lime and will produce irritation when it comes in to contact with mucous membrane. *Kadunkara Chunnam* is highly alkaline caustic and blisher forming.

Preservation and storage:

Chunnam should be kept in dry well stoppard glass bottles.

Uses of Chunnam

Generally chunnams are acclaimed as best possible medicaments in chronic degenerative human ailments with various adjuvants. Chunnam of salt peter, which is called as vediuppu chunnam, is used extensively by present day physicians for urinary calculi, and in some types of malignant and non-malignant tumours.

Chunnam of borax which is called as *venkara chunnam* are highly preferred in metabolic disorders of liver and spleen diseases. Many of these chunnam preparations play a vital role in the process of higher order medicines called Kattu (solidified metals). One of the best examples is solidified mercury called sootha kattu.

Among various chunnam preparations a very few preparations are considered very indispensable as medicine and in medicine preparation, are termed as Guru chunnam, Very few dings like gold, silver, copper sulphate, fuller's earth chunnam preparations are considered as gold chunnam and termed as thanga guru, velli guru, thurusu guru, muppu guru respectively

Among the many uniqueness of Siddha medicine, kayakalpam or rejuvenation therapy is its epitome. Food and life style modifications with ding therapy are indicated in this line of treatment. *Muppu chunnam* is basic and cardinal component in these preparations of antiageing processes and also in alchemical transformations.

Apart from this, *muppu chunnam* is also used as an adjuvant for many medicaments for chronic degenerative diseases and is a purifier of metals and minerals.

Many chunnam preparations of sal ammoniac (*kambi navachara chunnam*), Borneo camphor (*karpoora chunnam*), copper sulphate (*thurusu*), arsenic thalaga chunnam(*vanga chunnam*), copper (*thamira chunnam*), gold (*thalanga chunnam*), mercury (*rasa chunnam*), mercuric per chloride (*veera chunnam*), mica (*abrakam*), magnetic ore (*kantham iron aya chunnam*), arsenic oxide sulphide (*gowri*), sulphur (*gandagam*), cinnabar (*lingam*), zinc (*nagam*) are prepared with *muppu chunnam*.

It is worth mentioning that very small quantity of copper sulphate chunnam (*thurusu*) is added to extract juices or latex from very dry natured plants like *virali* (*Dodonea riscosa*), *kuppai meni* (*Acalypha indica*) and *erukku* (*Calotropis gigantica*).

4. MATERIALS AND METHODS

4.1 PREPARATION OF *VENKARA CHUNNAM*

DRUG SELECTION:

In this dissertation *VENKARA CHUNNAM* is taken as a trial drug for evaluate Lithotriptic, Diuretic, and Antispasmodic activity from the Siddha Literature *Anuboga vaithiya navaneetham, part 3 page no 25, 26,* Second Edition - 2002, Hakim P. Mohamed Abdulla Sahib, published by thamarai noolagam chennai 26

INGREDIENTS:

1. *Venkaram (Sodium bi borate)* – *1 balam (35gm)*
2. *Uppilangodi (pentatropis microphylla)* - *q.s*
3. *Murungai pattai (moringa oliefera)* -*q.s*

COLLECTION OF THE RAW DRUG AND MINERAL DRUG:

- The mineral drug *Venkaram was* purchased from Gopala Asan Naatu Marunthukadai, Nagercoil.
- The Uppilangodi was collected from poyapatti, Dharmapuri District.
- The Murangai pattai was collected from palayamkottai.

IDENTIFICATION AND AUTHENTICATION OF THE DRUG:

The identification of herbo mineral raw drug is authenticated by the faculties of PG Gunapadam Department, Government Siddha Medical College, Palayamkottai.

PURIFICATION OF THE DRUG:

All the herbomineral ingredients purified as per the traditional validation and illustration.

- ***Venkaram (borax)* -** The salt is washed in cow's dung solution.
- ***Uppilangodi (ambarvel)* –** wash with water until soil and dust particles removed.
- ***Murungai pattai (drumstick tree)* –** clean the bark with cloth and then cut down the outer layer of bark with the help of knife.

PREPARATION OF VENKARA CHUNNAM

Purified *venkaram* was kept in porcelain vessel and deeply soaked 2 inches (5cm) in uppilangodi juice for 3 days. Then it was dried in sunlight until the juice dry.

After that moringa bark was rubbed as butter consistency then this *venkaram* was covered with rubbed mass of moringa bark. Thus the moringa bark mass covered over 1 inch on *venkaram*. Then dry it in sunlight after that seven clay clothes was made around this mass, dried and subjected to pudam with 50 balam (1750 gram) varaties. After cooling *venkara chunnam* was taken out and powdered with the help of stone mortar.

STORAGE OF THE DRUG:

The prepared test drug was stored in a clean glass air tight container.

ADMINISTRATION OF DRUG:

Form of the medicine – *chunnam*

Dose – 2 – 4 *kundrimani* (260-520 mg) BID- morning and evening

Adjuvant – Honey, Ghee, butter, tender coconut

Shelf life – 500 years

INDICATION:

Kalladaippu (Renal Stone)

Neeradaippu,

Thasaiadaippu

INGREDIANTS OF VENKARA CHUNNAM

Figure No:1 VENKARAM

Figure No: 2: ***UPPILANGKODI*** **(*Pentatropis microphylla*)**

Figure No: 3: ***MURUNGAI PATTAI (Moringa oleifera)***

Figure: 4 PREPARATION OF VENKARA CHUNNAM

Purification of Venkaram

Soaked in uppilangodi Juice

Covered with moringa bark mass

Sealed Earthern crucible

Incineration process

After Incineration

Figure:5 End Process of Venkara Chunnam

4.2. STANDARDIZATION OF THE DRUG:

4.2.1 AS PER SIDDHA CLASSICAL LITERATURE:

Standardization of drug means confirmation of its quality and purity and detection of the nature of adulterant of various parameters like morphological, microscopic, physical, chemical and biological observations.

1) **Colour**:

   Mostly c*hunnam* is white in colour. *VENKARA CHUNNAM* is white in colour. It shows that the perfect colour of *chunnam*

2) **Odour**: Odourless

3) **Taste**:

   Properly prepared *chunnam* should be completely tasteless. If any taste present in *chunnam*, it indicates the preparation was not well prepared. It needs another *pudam* (incineration) process. A small amount of *chunnam* was kept in the tip of the tongue, which is tasteless and felt mild irritation due to its alkaline nature. The final product, *VENKARA CHUNNAM* was analysed as per Siddha classical standardization methods.

4) **Lustre**:

   If any glowing particles seen in the *chunnam*, it shows that the drug is not prepared properly and possess unchanged substances like metals and other toxic substances. So, there should be no glowing particles present in the properly prepared *chunnam*. The *VENKARA CHUNNAM* was taken in a petri dish and observed for any lustre in daylight via magnifying glass. No lustre was observed in the *parpam*.

5) **Finger print test:**

   Well prepared *chunnam* should be very fine. A pinch of *VENKARA CHUNNAM* was taken and rubbed in between the thumb and index finger. It enters into the lines of the fingers. It confirmed the fineness of *chunnam.*

6) **Floating on water:**

   A pinch of *VENKARA CHUNNAM* was sprinkled over the water in a glass container. The *chunnam* particles did not sink but floated on the water surface. It indicates the lightness of *VENKARA CHUNNAM*.

   Based on the above results it was suitable for further analysis.

7) **Colour change:**

   The chunnam was mixed with turmeric powder and add few drops of water. The yellow colour changed into red colour.

4.2.2. AS PER MODERN ASPECT:

4.2.2.1. PHYSICO CHEMICAL ANALYSIS:

Solubility test:

A. A little amount of the sample was taken in a clean, dry test tube and then shaken well with distilled water.

B. A little amount of the sample was taken in a clean dry test tube and then shaken well with Con. HCL and Con.H_2SO_4. Sparingly soluble character of the sample indicates the presence of Silicate.

Action on heat:

A small amount of the sample was taken in a clean dry test tube and heated gently. If strong white fumes evolve indicate the presence of Carbonate.

Flame test:

A small amount of the sample was taken in a clean dry watch glass. It was made into paste with concentrated HCL. And then it was introduced into non-luminous part of the Bunsen flame. If bluish green flame appears, it indicates the presence of copper.

Ash test:

A small amount of sample was mixed with the cobalt nitrate solution. A filter paper was soaked into the mixture. Then it was introduced into the Bunsen flame and ignited. If yellow colour flame appears, it reveals the presence of sodium.

Particle size:

25 gm of *VENKARA CHUNNAM* was taken in a sieve (sieve no: 200). It was shaken rotator vertically and horizontal direction by tapping on a hard surface for not less than thirty minutes. The amount of remnants was weighed accurately.

Loss on Drying (Indian Pharmacopoeia, 1996):

Loss on drying is the loss of percentage w/w resulting from water and volatile matter of any kind that can be driven off under a specified condition. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of the drugs.

Determination of Loss on drying:

The Loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. A glass stopper and a shallow weighing bottle were weighed accurately and the quantity

of the sample as specified was transferred to the bottle covered and weighed. The sample was distributed evenly and the bottle was placed in the drying chamber. The sample was then dried for a specific period of time, and the bottle was removed from the chamber and allowed to cool at room temperature in desiccators before weighing. The percentage of loss on drying (1.3%) was within acceptable range (5%-8%), thus implying that the formulation can be stored for a long period and would not easily be attacked by microbes.

Ash Value (Indian Pharmacopoeia, 1996):

The ash yielded by an organic compound measures the amount of inorganic matter present as impurity. Ash values are helpful in determining the quality and purity of crude drugs in the powder form. Ash determination judges the identity and cleanliness of drugs and provides information regarding adulteration with inorganic matter.

Determination of Total Ash Value:

Total ash includes physiological ash, which is derived from the medicine and non-physiological, which is often from environment contaminations such as sand and soil. 2 g of powdered drug was accurately weighted in a silica crucible which was previously ignited and weighed. The powdered drug was spread as a layer on the bottom of the crucible. It was then incinerated at a temperature not exceeding 450^{o}C until free from carbon. The crucible was then cooled and weighed.

Water soluble Ash:

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5 minutes with 25ml water. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15 minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained for determination of weight of insoluble ash. The weight of the water soluble ash is determined by subtracting the weight of insoluble ash from the weight of total ash.

Determination of Acid insoluble Ash:

Total Ash content alone is not sufficient to reflect the quality, thus the acid insoluble ash of the drug is quite important. The ash obtained in the determination of total ash, was boiled with 25 ml HCL for 5minuts and the insoluble ash was collected in a clean filter paper and washed with hot water. The insoluble ash was then transferred to a pre weighed silica crucible, ignited cooled and weighed.

Determination of extractive value:

Alcohol soluble extractive value:

3g of test drug powder is weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 18 hours. The solution is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

Water soluble extractive value:

3g of test drug powder is weighed and macerated with chloroform and water, respectively, at 80^{o}C for 24 hrs. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 24 hrs then filtered. The solution from both chloroform and water respectively is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

4.2.2.2. MICROBIOLOGICAL LIMIT TEST

Evaluation of total aerobic bacterial count

1. Preparation of Sample for Experimental Work

Weighed 10 gm of the homogenized drug sample aseptically and dissolved in 10 ml of sterile water and made up to 100 ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH7.0)

2. Serial dilution of Sample

A serial dilution is the dilution of a sample, in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile distilled water and mixed it well. This dilution was denoted as 10^{-1} dilution. From this dilution, one ml was taken from that mixture is added to 9 ml, and designated as 10^{-2} dilution. The same procedure was repeated up to $10^{-}4$.

3. Isolation of Total Viable Aerobic Microbial Count

Isolation of Bacteria by Plate Count Method

In this test, the bacteria in sample were made to grow as colonies, by inoculating a known volume of sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium) in petridish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45^{O}C, approximately 15 to 20 ml of medium was

poured into a sterile Petri dish under aseptic condition and left to solidify for 15 minutes. After solidification, each plate was smear with 0.1 ml of sample from the dilution of 10^{-1} and 10^{-2}. After inoculations, all the plates were incubated at 37^{o}C for 24 hours. After incubation, the bacterial colonies were developed as visible to the naked eye and the number of colonies on a plate was counted using Quebec Colony Counter. Plates with an average of from 30 to 300 colonies of the target bacterium were selected for colony count. Because of the statistical problems, plates with lowerthan 30 colonies greater than 300 colonies were rejected

Composition of Nutrient Agar Media

Peptone	:	5.0 gm
Sodium chloride	:	5.0 gm
Beef extract	:	1.5 gm
Yeast extract	:	1.5 gm
Agar	:	15.0 gm
Distilled water	:	1000 ml
pH (at 25°C)	:	7.4±0.2

Isolation of Fungi

From each of the above prepared samples, 0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25°C). After incubation, the fungal colonies were observed and calculated.

Composition of SDA

Dextrose	:	40 gm
Peptone	:	10 gm
Agar	:	15 gm
Distilled water	:	1000 ml

4. Evaluation of Antimicrobial Activity of Drug

Antimicrobial activity was performed by agar well diffusion method on agar.

Preparation of drug extracts solutions for the experiment

The dried drugs were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 10, 20 and 30µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

Procedure for the Agar Well Diffusion Test

The antibacterial screening of the drugs were carried out by determining the zone of inhibition using agar well diffusion method. All the drug extracts were tested against four pathogenic bacterial strains of gram positive and gram negative organism by agar well diffusion method.

Bacterial Inoculums Preparation

Inoculums of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus substilis* were prepared in nutrient broth medium and kept for incubation at 37°C for 8 hrs.

Agar well-diffusion method

This method was followed to determine the antimicrobial activity. Muller-Hinton Agar media plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. After inoculation, wells with the size of 10 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution of each drug extract was prepared at a concentration of 1 mg/ml in water. About 100 µl of different concentrations of drug solvent extracts were added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

Composition of Muller Hinton Agar Media

Beef Extract	:	02.00 gm
Acid Hydrolysate of Casein	:	17.50 gm
Starch	:	01.50 gm
Agar	:	17.00 gm

5. Evaluation of Specified Microorganisms

Isolation & Identification of *Escherichia coli*

One ml of the prepared sample was added in a sterile screw-capped container containing 50 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 5 ml of Mac- Conkey broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours.

Secondary Test

From the primary test, 1.0 ml of the enrichment culture was taken and transferred aseptically in to 5 ml of peptone water. It was then incubated in a water-bath at 43.5º to 44.5º C for 24 hours and observed the tubes for acid and gas. Then, the culture was subjected to biochemical tests of imvic and the results were observed and correlated.

Alternative test

It was done by a loop full of enriched culture in the primary test was streaked on a sterile Mac-Conkey agar medium. Then, the plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the pink or brick red color colonies were examined and transfer them individually into the surface of Eosin Methylene Blue agar medium (EMB), on Petri dishes. Inoculated plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the colonies on medium were checked for their color appearance like green metallic sheen under reflected light. The colonies were subjected to confirmation by further suitable cultural and biochemical tests.

Components of Eosin Methylene Blue Agar Media

Pancreatic digest of gelatin	:	10.0 g
Dibasic potassium phosphate	:	2.0 g
Lactose	:	10.0 g
Eosin Y	:	400 mg
Methylene blue	:	65 mg
Agar	:	15.0 g
Distilled water	:	1000 ml

Isolation & Identification of *Salmonella* sp

One ml of the prepared sample was added in a sterile screw-capped container containing 100 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 10 ml of Selenite F broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours. After incubation, the culture was subcultured on two of the agar media namely Bismuth sulphate

agar and Deoxy cholate citrate agar and incubated the plates at 36º to 38º for 18 to 24 hours. After incubation, colonies were observed on the medium and confirmed the genus *Salmonella* based on guidelines.

Secondary test

The suspected colonies of the primary test were subcultured on the slant of triple sugar-iron agar in test tube and in urea broth. Both media were incubated at 37ºC for 24 hours. After incubation, the results were observed according to the development of color change and acid / gas in media. The presence of Salmonella was confirmed by agglutination tests.

Composition of *Salmonella Shigella* Agar Media

Beef Extract	:	5.0 gm
Enzymatic Digest of Casein	:	2.5 g
Enzymatic Digest of Animal Tissue	:	2.5 gm
Lactose	:	10 gm
Bile salts	:	8.5 gm
Sodium Citrate	:	8.5 gm
Ferric Citrate	:	1.0 gm
Brilliant Green	:	0.00033 gm
Neutral Red	:	0.025
Agar	:	13.5 gm
Distilled water	:	1000 ml

Isolation and Identification of *Pseudomonas aeruginosa*

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into 100 ml of fluid soyabean-casein digest medium and mixed well. The inoculated tubes were incubated at 37º C for 24 hours. After incubation, the growth of bacteria was checked. From this, a loop full of culture was streaked on the surface of Cetrimide agar medium and Pseudomonas Isolation Agar medium and incubated at incubated at 37º C for 24 hours. After incubation, the colonies from the agar surface of these two media were checked for detection of fluorescein and pyocyanin.

Composition of Cetrimide Agar Media

Pancreatic digest of gelatin	: 20.0 g
Magnesium chloride	: 1.4 g
Potassium sulphate	: 10.0 g
Cetrimide	: 0.3 g

Agar	: 13.6 g
Glycerin	: 10.0 g
Distilled Water t	: 1000 ml

Isolation and Identification of *Staphylococcus aureus*

From the above prepared enrichment culture, a loop full of culture was taken and transferred aseptically on Mannitol salt agar and incubated at 37º C for 24 hours.. After incubation, the colonies were subjected to confirmation by hem agglutination test.

Composition of Mannitol Salt Agar Media

Pancreatic digest of gelatin	: 5.0 g
Peptic digest of animal tissue	: 5.0 g
Beef extract	: 1.0 g
D-Mannitol	: 10.0 g
Sodium chloride	: 75.0 g
Agar	: 15.0 g
Phenol red	: 25 mg
Distilled Water	: 1000 ml

4.2.2.3 BIO CHEMICAL ANALYSIS:

Preliminary Basic and Acidic radical studies:

Preparation of the extract:

100gms of VENKARA CHUNNAM is weighed accurately and placed into a 250ml clean beaker and added a few drops of concentration Hydrochloric acid and evaporated it well. After conc. nitric acid and evaporated it well. After cooling the content add 20ml of distilled water and dissolved it well. Then it is transferred to 100ml volumetric flask and made up to 100ml with distilled water mix well, filter it. Then it is taken for analysis.

QUALITATIVE ANALYSIS FOR BASIC RADICALS:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

Test for Iron (Ferrous):

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for Zinc:

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc.

QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitricacid. Formation of yellow precipitate indicates the presence of phosphate.

Test for Carbonate:

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for albumin:

The extract is treated with Esbach`s reagent. Formation of yellow precipitate indicates the presence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannicacid

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

Test for amino acid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

4.2.2.4. PHYTO CHEMICAL ANALYSIS

Analysis of Test Drug

Siddha preparation of *Venkara chunnam* was prepared and used for phytochemical analysis. Preliminary test, on siruneer kalluku kudineer was carried out for the presence of alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, phenolic compounds, proteins and free amino acids, flavonoids, lignin, fixed oils and fats. The methods adopted for the estimation are as follows:

Test for Alkaloids (Evans, 1997):

A small segment of *Venkara chunnam* was mixed separately with a few drops of dilute hydrochloric acid and filtered. The filtrates were tested carefully with various alkaloidal reagents asfollows:

Mayer's test (Evans,1997):

To a few ml of filtrate, a drop of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates that the test as positive.

Hager's test (Wagner et al.,1996):

To a few ml of filtrate, one to 2ml of Hager's reagent is added. A prominent yellow precipitate indicates the test as positive.

Dragendorff's test (Waldi,1965):

To a few ml of filtrate, one to 2ml of Dragendorff's reagent is added. A prominent yellow precipitate indicates the test as positive.

Test for Carbohydrates (Ramakrishnan et al., 1994) :

A small quantity of *Venkara chunnam* was dissolved separately in 5ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates. Filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol solution and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of 2 layers shows the presence of carbohydrates.

Test for Glycosides:

Siddha preparation of *Venkara chunnam* was hydrolyzed with hydrochloric acid for few hour on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

a)Legal's Test:

To the hydrolysate, one ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides and aglycones.

(b) Borntrager's Test:

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink color, shows the presence of glycosides (Evans, 1997).

Test for Phytosterols (Finar, 1986)

a)Liebermann Burchard Test:

Small amount of *Venkara chunnam* was dissolved with 3ml of acetic anhydride, a few drops of glacial acetic acid and followed by the addition of few drops of concentrated sulphuric acid. Appearance of bluish green color shows the presence ofphytosterols.

b.Salkowski Test:

Small quantities of *Venkara chunnam* were dissolved in chloroform separately. This chloroform solution was added with few drops of concentrated sulphuric acid. The appearance of bluish green color shows the presence of phytosterols

Test for Saponins (Kokate, 1999)

Frothing Test:

Venkara chunnam was diluted separately with 20ml of distilled water and it was agitated on a graduated cylinder for 15min. Absence of the foam formation shows the devoid of saponins.

Test for Phenolic Compounds and Tannins (Mace, 1963) :

Small quantities of *Venkara chunnam* was dissolved separately in water and tested for the presence of phenolic compound and tannins. In the process of testing and treating, the following observations were noted:

a) Dilute ferric chloride solution (5%) gives a dark greencolor.

b) 10% aqueous potassium dichromate solution gives yellowish brownprecipitate.

c) 10% lead acetate solution gives a whiteprecipitate.

Test for Proteins and FreeAminoAcids(Fisher,1968; Ruthmann,1970)

Small quantities of *Venkara chunnam* was dissolved in few ml of water and the following reaction were carried out

Millon's Test:

To 2ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins (Rasch and Swift,1960).

Ninhydrin Test:

To 2ml of filtrate 2 drops of ninhydrin solution was added. A characteristic purple color indicates the presence of amino acids (Yasma and Ichikawa,1953).

Biuret Test:

An aliquot of 2ml of filtrate was treated with a drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, Pink color in the ethanol layer indicates the presence of protein (Gahan,1984)

Test for Flavanoids

Shinoda's Test:

Small quantity of *Venkara chunnam* was treated with alcohol to that a piece of magnesium was added followed by an addition of concentrated hydrochloric acid drop wise and heated. Appearance of magenta color shows the presence of flavanoids (Harborne,1984).

Florescence Test:

Small quantity of *Venkara chunnam* was dissolved separately in alcohol and a drop of that extract was placed on Whatman filter paper and observed under UV light. Florescence indicates the presence offlavanoids.

Tests for Lignin:

Small quantities of *Venkara chunnam* was dissolved separately in few ml of alcoholic solution of hydrochloric acid and phloroglucinol gives red color, which shows lignin ispresent.

Tests for Fixed oils and Fats:

Spot Test:

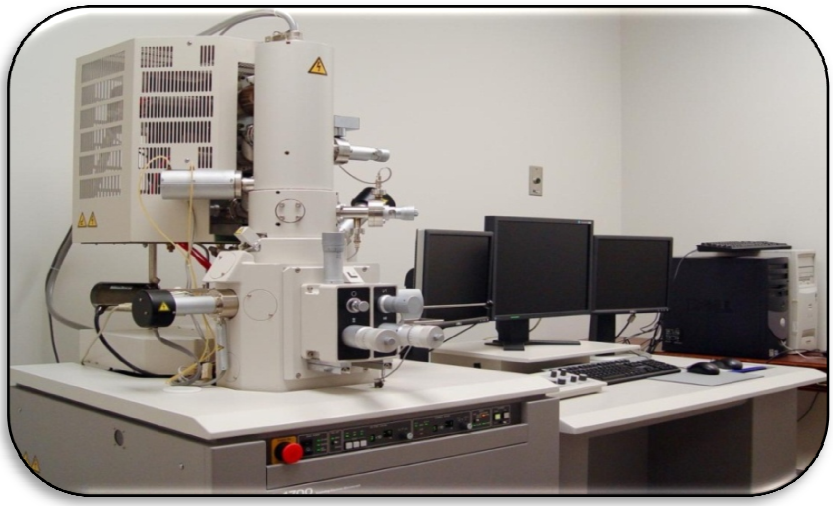
A small quantity of *siruneer kalluku kudineer* was placed between 2 filter papers. Oil stains produced with any extract shows the presence of fats and fixed oils

SaponificationTest:

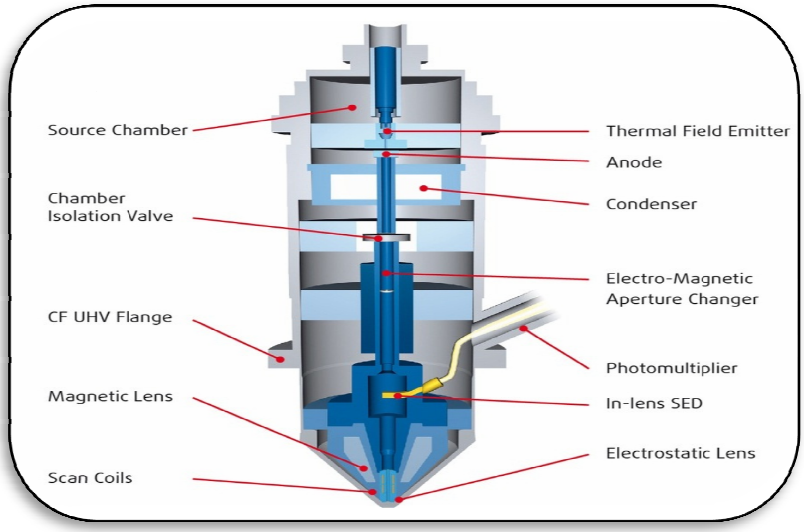
A small quantity of *Venkara chunnam* was treated with few drops of 0.5N alcoholic potassium hydroxide along with 2 to 3 drops of phenolphthalein. Later the mixture is refluxed for about 2h. Soap formation indicates the presence of fats and fixed oils.

4.2.2.5.INSTRUMENTAL ANALYSIS

1. SEM *(SCANNING ELECTRON MICROSCOPE)*



MECHANISM



Introduction:

The scanning Electron Microscope is one of the most versatile instruments available for the examination and analysis of the micro structural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5nm (50degreeA) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5nm

(25 degree A). Any solid material can be studied. Sample size is limited to specimens less than about 10μm in diameter

Principle:

The beam is then rested over the specimen in synchronism with the beam of a cathode ray tube display screen. The elastically scattered secondary electrons are emitted from the sample surface and collected by a scintillator, the signal from which is used to modulate the brightness of the cathode ray tube. In this way the secondary electron emission from the sample is used to form an image on the CRT display screen. (Goldstein, et. al., 1992)

Procedure:

An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction).So when an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron) enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen is sufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons Colliding with elemental components of the specimen and releasing

secondary electrons also increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface. Consequently, the electron emission yield decreases. Therefore, the peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field.

Applications:

The SEM is capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in *Siddha* system as well as other fields. The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo. Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical *Siddha* herbo mineral drug *VENKARA CHUNNAM*. SEM results of *VENKARA CHUNNAM* were represented in results section.

2. FT-IR (Fourier Transform Infrared Spectroscopy)

FTIR INSTRUMENT

Introduction:

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy. In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle:

Infra-red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

Source	:	Nernst Glower
Beam splitter	:	It is made up of a transparent material. Thin films of Silicon deposited on Potassium bromide (KBr)
Bromide (KBr) Detectors:		Deutrated TriGlycine Sulphate (DTGS).
Scan Range	:	MIR 450to 4000 cm-1
Resolution	:	4.0 cm- 1
Sample required	:	50mg, solid or liquid
Sampling Techniques:		There are a variety of techniques for sample preparation physical form of the sample to be analyzed.
Solid	:	KBr or Nujol mull method.
Liquid	:	Csl / TIBr Cells
Gas	:	Gas cells

Measurements Techniques:

The procedure for recording the %T or %A is as follows:

- Air is first scanned for the reference and stored. The sample is then recorded and finally the ratio of the sample and reference data is computed to give required %T or %A at various frequencies.
- Study of substances with strong absorbance bands and weak absorbance bands as well as possible.
- Small amount of samples are sufficient
- High resolution is obtained.

Procedure:

- Typically, 1.5 mg of protein, dissolved in the buffer used for its purification were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.

- Then, 300 Al of 20 mm buffer, prepared in H_20 or $2H_20$, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the Ph meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the Tris buffer.
- The washings took 24 h, which is the time of contact of the protein with the 2H20 medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.
- The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuterated triglycinesulfate detector.

1. At least 24 h before, and during data acquisition, the spectrometer were continuously purged with dry air at a dew point of 40^{o}C. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5^{o}C steps from 20 to 95^{o}C.
2. Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of H_20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of $2H_20$ was adjusted to the removal of the$2H_20$ bending absorption close to 1220 cm.

KBr Method

1. The sample is grounded using an agate mortar and pestle to give a very fine powder.
2. The finely powder sample is then mixed with about 100mg dried KBr salt.
3. The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

Nujol Mull Method:

1. The sample is ground using an agate mortar and pestle to give a very fine powder.

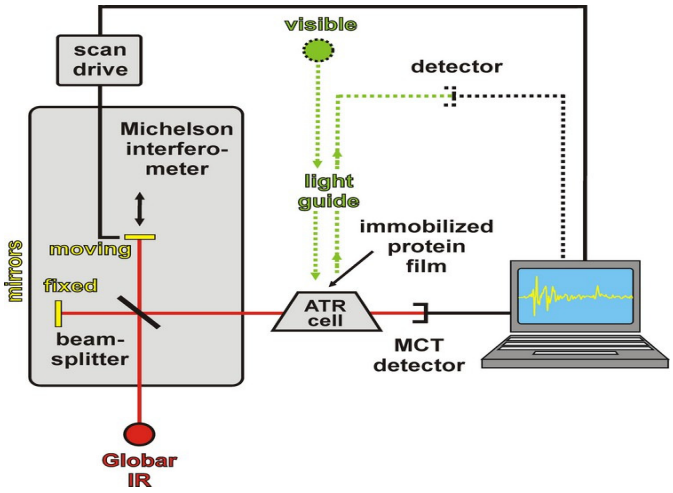
2. A small amount is then mixed with nujol oil to give a paste and this paste is then applied between two sodium chloride plates.
3. The plates are then placed in the instrument sample holder ready for scanning.

Liquids:

1. Viscous liquids can be smeared in the cell and directly measured.
2. For dilute solutions, liquid cells and variable path length cells are employed.

Applications:

Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH2, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.



Mechanism of FTIR analyzer

Analytical Capabilities:

1. Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond.
2. Especially capable of identifying the chemical bonds of organic materials
3. Detects and identifies organic contaminants.
4. Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions
5. Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm^3 or sometimes sub monolayer .Useful with solids, liquids, or gases.

3. ICP-OES *(INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)*

ICP-OES *(INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)*

Manufacturer: Perkin Elmer

Model : Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer *(ICP)*

Principle :

An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone *(8,000– 10,000ºC).* The analysts are heated *(excited)*to different (atomic and/or ionic) states and produce characteristic optical emissions *(lights).* These releases are separated based on their respective wavelengths and their strengths are measured *(spectrometry).* The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification is an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant *(such as the concentration of ferrous iron or ferric iron)*, only total essential concentration is analysed by ICP-OES.

Application:

The analysis of major and minor elements in solution samples

Objectives:

a) Determine elemental concentrations of different metals.
b) Learn principles and operation of the ICP-OES instrument
c) Develop and put on a method for the ICP-OES sample analysis
d) Enhance the instrumental conditions for the analysis of different elements
e) probes the outer electronic structure of atoms

Mechanism:

In plasma emission spectroscopy *(OES),* a sample solution is presented into the core of inductively coupled argon plasma *(ICP),* which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values. The Inductively Coupled Plasma Optical Emission Spectrometric *(ICP-OES)* analysis was done in Saif, IIT Madras, and Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis.

100 mg *"VENKARA CHUNNAM"*was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowedfor few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

4. X-RAY POWDER DIFFRACTION

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined. Max von Laue, in 1912, discovered that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing.

X-ray diffraction is based on constructive interference of monochromatic X- rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law (nX=2d sin 9). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X- rays are then detected, processed and counted. By scanning the sample through a range of 29angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacing's allows identification of the mineral because each mineral has a set of unique d-spacing's. Typically, this is achieved by comparison of d-spacing's with standard reference patterns.

All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation beyond this.

X-ray Powder Diffraction (XRD) Instrumentation

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder, and an X-ray detector.

X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most

common being Ka and Kp. Ka consists, in part, of Ka1 and Ka2. Ka1 has a slightly shorter wavelength and twice the intensity as Ka2. The specific wavelengths are characteristic of the target material (Cu, Fe, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. Ka1 and Ka2 are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with CuKa radiation = 1.5418A. These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle 9 while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 29. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 29 from ~5° to 70°, angles that are present in the X-ray scan.

Applications

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include:

1. characterisation of crystalline materials
2. identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
3. determination of unit cell dimensions
4. measurement of sample purity

With specialized techniques, XRD can be used to:

1. determine crystal structures using Rietveld refinement
2. determine of modal amounts of minerals (quantitative analysis)

3. characterize thin films samples by:
4. determining lattice mismatch between film and substrate and to inferring stress and strain
5. determining dislocation density and quality of the film by rocking curve measurements
6. measuring superlattices in multilayered epitaxial structures
7. determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
8. make textural measurements, such as the orientation of grains, in a polycrystalline sample

Strengths and Limitations of X-ray Powder Diffraction (XRD)

Strengths

1. Powerful and rapid (< 20 min) technique for identification of an unknown mineral
2. In most cases, it provides an unambiguous mineral determination
3. Minimal sample preparation is required
4. XRD units are widely available
5. Data interpretation is relatively straight forward

Limitations

1. Homogeneous and single phase material is best for identification of an unknown
2. Must have access to a standard reference file of inorganic compounds (d-spacings, hkls)
3. Requires tenths of a gram of material which must be ground into a powder
4. For mixed materials, detection limit is ~ 2% of sample
5. For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated
6. Peak overlay may occur and worsens for high angle 'reflections'
7. User's Guide - Sample Collection and Preparation
8. Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.
9. Obtain a few tenths of a gram (or more) of the material, as pure as possible Grind the sample to a fine powder, typically in a fluid to minimize inducing

extra strain (surface energy) that can offset peak positions, and to randomize orientation. Powder less than ~10 ^m(or 200-mesh) in size is preferred

10. Place into a sample holder or onto the sample surface
11. Packing of fine powder into a sample holder.
12. Smear uniformly onto a glass slide, assuring a flat upper surface
13. Pack into a sample container
14. Sprinkle on double sticky tape
15. Typically the substrate is amorphous to avoid interference
16. Care must be taken to create a flat upper surface and to achieve a random distribution of lattice orientations unless creating an oriented smear.
17. For analysis of clays which require a single orientation, specialized techniques for preparation of clay samples are given by usages.
18. For unit cell determinations, a small amount of a standard with known peak
19. positions (that do not interfere with the sample) can be added and used to
20. Correct peak positions.

Data Collection, Results and Presentation

Data Collection The intensity of diffracted X-rays is continuously recorded as the sample and detector rotate through their respective angles. A peak in intensity occurs when the mineral contains lattice planes with d-spacings appropriate to diffract X-rays at that value of 9. Although each peak consists of two separate reflections (Ka1 and Ka2), at small values of 29 the peak locations overlap with Ka2 appearing as a hump on the side of Ka1. Greater separation occurs at higher values of 9. Typically these combined peaks are treated as one. The 21 position of the diffraction peak is typically measured as the center of the peak at 80% peak height.

Data Reduction

Results are commonly presented as peak positions at 29 and X-ray counts (intensity) in the form of a table or an x-y plot. Intensity (I) is either reported as peak height intensity, that intensity above background, or as integrated intensity, the area under the peak. The relative intensity is recorded as the ratio of the peak intensity to that of the most intense peak (relative intensity = I/I1 x 100).

Determination of an Unknown

The d-spacing of each peak is then obtained by solution of the Bragg equation for the appropriate value of 1. Once all d-spacings have been determined, automated search/match routines compare the ds of the unknown to those of known materials. Because each mineral has a unique set of d-spacings, matching these d-spacings provides an identification of the unknown sample. A systematic procedure is used by ordering the d-spacings in terms of their intensity beginning with the most intense peak. Files of d-spacings for hundreds of thousands of inorganic compounds are available from the International Centre for Diffraction Data as the Powder Diffraction File (PDF). Many other sites contain d-spacings of minerals such as the American Mineralogist Crystal Structure Database. Commonly this information is an integral portion of the software that comes with the instrumentation.

POWDER X-RAY DIFFRACTO METER

4.3 TOXICOLOGICAL STUDIES

4.3.1 ACUTE TOXICITY STUDY OF *VC* IN FEMALE WISTAR ALBINO RATS.

The aim of this Study is to evaluate the toxicity of the test substance *VENKARA CHUNNAM,* when administered orally to Female Wistar albino Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

OECD Guidelines No 423. (Organization for Economic Cooperation and Development)

Study Design and Controls:

1. Female Wistar albino Rats in controlled age and body weight were selected.
2. The test drug VC was administered at 5 mg/kg, 10 mg/kg, 300 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight of animal as suspension along with water.
3. The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

1. ANIMALS

Supply

A total of 15 Female Wistar albino Rats with an approximate age of 6 weeks and purchased from Trivandrum medical college, Trivandrum. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female Wistar Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

Housing

The Female Wistar albino Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 3 rats of the same sex and treatment group.

Each cage was identified by a card, color coded according to the dose level. This card stated the cage number, number and sex of the animals it contained, Study number, test substance code, administration route, dose level and Study Director's name, date of the arrival of the animals and initiation of treatment.

The temperature and relative humidity were continuously monitored. Lighting was controlled to supply 12 hours of light (7:00 to 19:00 hours) and 12 hours of dark for each 24-hour period.

The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

2. DIET

All the rats had free access to a pelleted rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants.

Water

The water was offered adlibitum in bottles.

3. ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The Female Wistar albino Rats belonging to the control group were treated with the vehicle (Water) at the same administration volume as the rest of the treatment groups.

a) Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Numbering and Identification

Group No	Animal Marking
1	Head
2	Body
3	Tail

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals

Numbering and Identification

Cage No	Group No	Animal Marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

b) Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

Table no - 2 Doses

GROUP	DOSE
Group-I	5 mg/kg
Group-II	50 mg/kg
Group-III	300 mg/kg
Group-IV	1000 mg/kg
Group-V	2000 mg/kg

The test item was administered as single dose. After single dose administration period, all animals were observed for 14days.

Dose Preparation

VC was added in distilled water and completely dissolved to form oral for administration. The dose was prepared of a required concentration before dosing by dissolving, in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

c) Administration

The test item was administered orally to each Female Wistar rats as single dose using a needle fitted onto a disposable syringe of appropriate size at the

following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

d) Observation period

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cageside observation was included changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

4. Mortality and Morbidity

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days.

4.3.2 SUB-ACUTE TOXICITY STUDY IN WISTAR RATS TO EVALUATE TOXICITY PROFILE OF VENKARA CHUNNAM (VC)

1. Objective

The objective of this 'Sub-Acute Toxicity Study of *VC* on Wistar Rats' was to assess the toxicological profile of the test item when treated as a single dose daily. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

2. Test Guideline Followed

OECD (Organization for Economic Cooperation and Development) 407 Method - Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

3. Test Item Detail

Name: *VENKARA CHUNNAM*

4. Test System Detail

The study was conducted on 5 male 5 female Wistar rats for each group. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *adlibitum* in the Animal at Arulmigu Kalasalingam College, Krishnankoil. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

5. Acclimatization

The animals were selected after veterinary examination by the veterinarian. All the selected animals were kept under acclimatization for a week.

6. Randomization& grouping

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 5 male and 5 female animals per group.

7. Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table no – 3 Numbering and Identification acute

Group No (CONCENTRATION/ DOSE)	**Animal Marking**
1. CONTROL	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
2. LOW DOSEOF *VC*2ml/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
3. MIDDLEDOSEOF *VC*5ml/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
4. HIGH DOSEOF *VC*10ml/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)

Numbering and Identification

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals:

Table no – 4 Numbering and Identification subacute

Cage No	**Group No CONCENTRATION/ DOSE**	**Animal Marking**	**Sex**	**No of rats**
1	CONTROL	H,B,T,HB,NM H,B,T,HB, NM	Male Female	5 5
2	LOW DOSE OF *VC* 2ml/kg	H,B,T,HB,NM H,B,T,HB,NM	Male Female	5 5
3	MIDDLEDOSE OF *VC* 5ml/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female	5 5
4	HIGH DOSE OF *VC* 10ml/kg	H,B,T,HB,NM H,B,T,HB ,NM	Male Female	5 5

8. Husbandry

8.1 Housing

The Wistar rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 10 rat of the different sex and treatment group.

8.2 Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22 ± 3^{0}C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

8.3 Feed & feeding schedule

Arulmigu Kalasalingam College of Pharmacy Krishnankoil. Feed was provided *adlibitum throughout* the study period, except overnight fasting (18-20 hours) prior to dose administration. After the substance has been administered, food was withheld for a further 3-4 hours.

8.4 Water

The water was offered *adlibitum* in bottles. There was periodically analyzed to detect the presence of possible contaminants

8.5 Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted on to a disposable syringe of approximate size at the following different doses.

The test item was administered as single dose daily. After single dose administration period, all animals were observed for 28 days.

Dose Preparation

VC was added in distilled water and completely dissolved for oral administration. The dose was prepared of a required concentration before dosing by dissolving *VC* in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

8.6 Administration

The test item was administered orally to each rat as single dose using a needle fitted on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

9. OBSERVATIONS

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior.

9.1. Clinical signs of toxicity

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill- health or behavioral changes. Clinical signs of toxicity daily for 28 days.

9.2. Food intake

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

9.3. Water intake

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

9.4 Bodyweight:

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection:

Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing

Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 6 rats from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN etc……...

Hematology

The following hematological parameters were analysed using Autoanalyser

Hb : Haemoglobin (g %)

PCV : Packed Cell Volume

WBC : White Blood Corpuscles ($x10^3$/cmm)

RBC : Red Blood Corpuscles ($x10^6$/cmm)

Blood Platelet count :($x10^3$/cmm)

Differential WBC count:

N : Neutrophils (%)

L : Lymphocytes (%)

M : Monocytes (%)

E : Eosinophils (%)

RDW : Red Cell Distribution Width.

MPV : Mean Platelet Volume

Clinical Biochemistry:

The following clinical Bio parameters were analysed using Auto analyser

Total serum protein (g/dl)

ALT/SGPT : Alanine amino transferase (U/L)

AST/SGOT : Aspartate amino transferase (U/L)

ALP : Alkaline serum phosphatase (U/L)

CHL : Cholesterol (mg/dL)

HDL : High density lipoprotein

TG : Triglyceride

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 18 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights:

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

4.4 PHARMACOLOGICAL STUDY

4.4.1 LITHOTRIPTIC ACTIVITY

EVALUATION OF LITHOTRIPTIC (ANTILITHIATIC) EFFECT OF *VC* ON 1% ETHYLENE GLYCOL INDUCED LITHIASIS IN ALBINO RATS

AIM:

Aim of the study is to evaluate the lithotriptic activity of *venkara chunnan* in male Wistar albino rats.

Materials and methods

Animal selection

Wistar albino rat of either sex weigthing between 25 and 30g were selected and healthy adult male Wistar albino rats weigthing between 150 and 200g were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25±2°C) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and ad libitum. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC).

Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats. Animals were divided into five groups containing six animals in each.

TREATMENT PROTOCOL

The grouped animal's received the treatment as follows

Group I Received normal diet and served as controls.

Group II Lithiatic control: The animals were given normal diet and 1% Ethylene glycol in drinking water for 28 days.

Group III Received 1% Ethylene glycol in drinking water and then treated with cystoneat a dose of 100mg/kg orally for 28 days.

Group IV Received 1% ethylene glycol in drinking water and then treated with *VC* at a dose of 200 mg/kg orally for 28 days

Group V Received 1% Ethylene glycol in drinking water and then treated with *VC* at a dose of 400mg/kg orally for 28 days.

Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 14th, and 28th day of calculi induction treatment. The volume and calcium content of urine were measured. Calcium in urine was estimated using kit by "COBAS MIRA PLUS" auto analyzer. Urine was analyzed for oxalate, magnesium, phosphate, uric acid, citrate and total protein.

Serum analysis

The blood was collected from the retro-orbital sinus under anesthetic condition and serum was separated by centrifugation at 10,000g for 10 min and analyzed for creatinine and uric acid. The creatinine kit (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kit (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid levels respectively.

Statistical analysis

The results were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Newmann keul's multiple range tests and $p < 0.05$ was considered significant.

4.4.2 DIURETIC ACTIVITY

EVALUATION OF DIURETIC ACTIVITY EFFECT OF VC ON MALE ALBINO RATS (LIPSCHITS ET AL METHOD)

AIM:

Aim of the study is to evaluate the diuretic activity of *venkara chunnan* in male Wistar albino rats

Material and Methods

Experimental animals

Healthy male albino rats weighing 180-200 g were used for the study. The animals were maintained in polypropylene cages of standard dimensions at a temperature of 37 ± 1°C and standard 12h : 12h day/night rhythm. The animals were fed with standard rodent pellet diet (Hindustan Lever Ltd.) and water *adlibitum*. Prior to the experiment, the animals were acclimatized to the laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

Drug Treatment

VC at the dose levels of 200, 400 and 600 mg/Kg body wt., p.o. was administered once daily for three consecutive days. Furosemide (20 mg/Kg; p.o.) was used as standard for diuretic activity. Control group of animals (n=6) received normal saline (10 ml/Kg)

Experimental design

The animals were divided into 5 groups of 6 rats each as follows; Group I: received only 10ml/kg normal saline Group II: received Furosemide 20 mg/kg, Group III: received *VC* 200 mg/kg body weight p.o., Group IV: received *VC* 400 mg/kg body weight p.o. and Group V: received *VC* 600 mg/kg body weight p.o.

Diuretic activity

Rats were fasted overnight and treated with vehicle, Furosemide and *VENKARA CHUNNAM* as stated above along with normal saline (10 ml/kg). The rats were placed in metabolic cages and the urine samples were collected for 24h, measured using a standard measuring cylinder. The amount of urine (in ml) collected for 24 h was compared and tabulated.

Natriuretic activity

Estimation of Sodium and Potassium content of the urine samples of all groups of animals were done by using a laboratory model flame photometer. The ratio of Na+/K+ is calculated for Natriuretic activity. A value greater than 2.0 indicates a favorable Natriuretic effect. Ratio greater than 10.0 indicates a potassium sparing effect. Ratio greater than 10.0 indicates a sparing effect.

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical comparisons were made by means of newmann keuls multiple range tests. P values smaller than 0.05 was considered as significant.

4.4.3 ANTI-SPASMODIC ACTIVITY

IN-VITRO ANTI-SPASMODIC ACTIVITY OF *VC* ON EXCISED GUINEA PIG (STUDENTS ORGAN BATH)

ISOLATION OF GUINEA PIG:-

Guinea pigs were anesthetized and sacrificed by cervical displacement followed by exsanguinations. The ileum was dissected out, immersed in Tyrode'ssolutionand cleaned off the mesentery. Respective segments of 2-3cm long were mounted in a 25ml tissue organ bath, filled with a mixture of 95% O2 and 5% CO2 and maintained at 37 o C. The composition of Tyrode's solution (in mm for l lit) was 9 mg KCl, 0.1 mg NaCl, 0.1mg NaHCO3, 0.42mg NaH2PO4, 0.6 mg Glucose and pH value was 7.4.

ANTI-SPASMODIC ACTIVITY ASSAY PROCEDURE:-

1. Firstly concentration dependent responses of acetylcholine were recorded (with dose of 0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml, 3.2ml) using Sherrington's recording drum with a frontal writing lever. Contact time of 60 sec, and base line of 30sec time cycle were opted for proper recording of the responses in presence of plane Tyrode's solution as stock-I solution.
2. Then same concentration dependent responses of acetylcholine (Ach) using same procedure for a mixture of Tyrode's solution+ Lantana camara extract (with a concentration of 1mg/ml) as a stock-II solution were recorded.
3. Lastly the same concentration dependent responses of Ach for a mixture of Tyrode's solution+ Atropine (as a standard antispasmodic agent) as a stock-III solution were recorded.

5. MICROBIOLOGICAL ANALYSIS

AIM:

Antimicrobial activity of *Venkara chunnam* was performed by agar well diffusion method on agar.

COMPOSITION OF MULLER HINTON AGAR MEDIA

Beef Extract	: 02.00 gm
Acid Hydrolysate of Casein	: 17.50 gm
Starch	: 01.50 gm
Agar	: 17.00 gm

PREPARATION OF DRUG EXTRACTS SOLUTIONS FOR THE EXPERIMENT

The dried drugs were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 10, 20 and 30µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

PROCEDURE FOR THE AGAR WELL DIFFUSION TEST

The antibacterial screening of the drugs were carried out by determining the zone of inhibition using agar well diffusion method. All the drug extracts were tested against four pathogenic bacterial strains of gram positive and gram negative organism by agar well diffusion method.

BACTERIAL INOCULUMS PREPARATION

Inoculums of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus substilis* were prepared in nutrient broth medium and kept for incubation at 37°C for 8 hrs.

AGAR WELL-DIFFUSION METHOD

This method was followed to determine the antimicrobial activity. Muller-Hinton Agar media plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. After inoculation, wells with the size of 10 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution of each drug 135 extract was prepared at a concentration of 1 mg/ml in water. About 100 µl of different concentrations of drug solvent extracts were added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

6. RESULTS AND DISCUSSION

The Siddha herbal drug *VENKARA CHUNNAM* had been subjected to various studies to establish its efficacy. Literary collections, physicochemical and elemental analysis, toxicological study, pharmacological study and clinical study are done to prove the activity of *VENKARA CHUNNAM in* lithontriptic, diuretic, anti-spasmodic (smooth muscle relaxant) activity.

STANDARDISATION OF THE TEST DRUG

Standardization of the drug is more essential to derive the efficacy and potency of the drug, which was analyzed by the various methods. The results of physicochemical and biochemical analysis have been done and tabulated. Pharmacological activity and toxicological results of the drug were derived. The results reveal the effectiveness of the trial drug *VENKARA CHUNNAM* has been proved by the following scientific parameters.

***SIDDHA* STANDARDIZATION METHODS:**

Siddhars used these following standardization methods to ensure the safety and efficacy of the chunnam. It shows the effectiveness of the drug.

Table No: 5 Results of Siddha standardization

S.NO	Parameter	Results of VC	Interpretation
1.	Colour	White	Indicates complete calcinations process
2.	Odour	Odourless	Indicates complete calcinations process
3.	Taste	Tasteles	Indicates complete calcinations process
4.	Finger Print Test	Impinged in the furrow of fingers	Indicates fine particles of powder.
5.	Floating on Water	Floats on water	Lightness of drug.
6.	Luster	Lusterless	No lowing particle seen. It indicates complete calcinations process
7.	Colour Cahnge	Red colour	Mixed with turmeric powder and add few drops of water. The yellow colour changed into red colour.

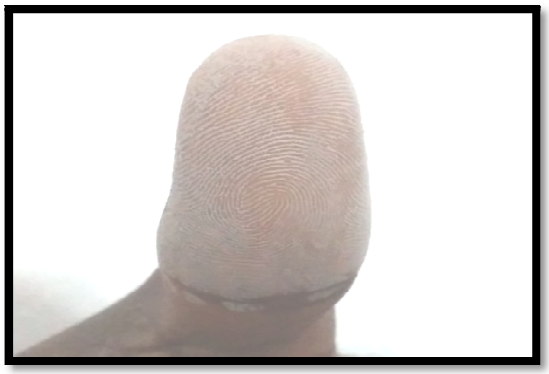
Interpretation

- The test drug VC being tasteless, odourless, lustreless and indicates complete calcinations process.
- It is an ideal colour for c*hunnam.* Without shining indicates no free form of metals present in that drug.
- The test drug which was float on water has less specific gravity, possesses specific gravity less than the water.
- On Finger print test, only the particles which are in micro fine size that can enter into the furrows of the finger print indicates.
- VC getting impinged in the furrows of finger print indicates pressure of micro fine particles and completes calcinations process.

STANDARDIZATION OF VC AS PER SIDDHA LITERATURE

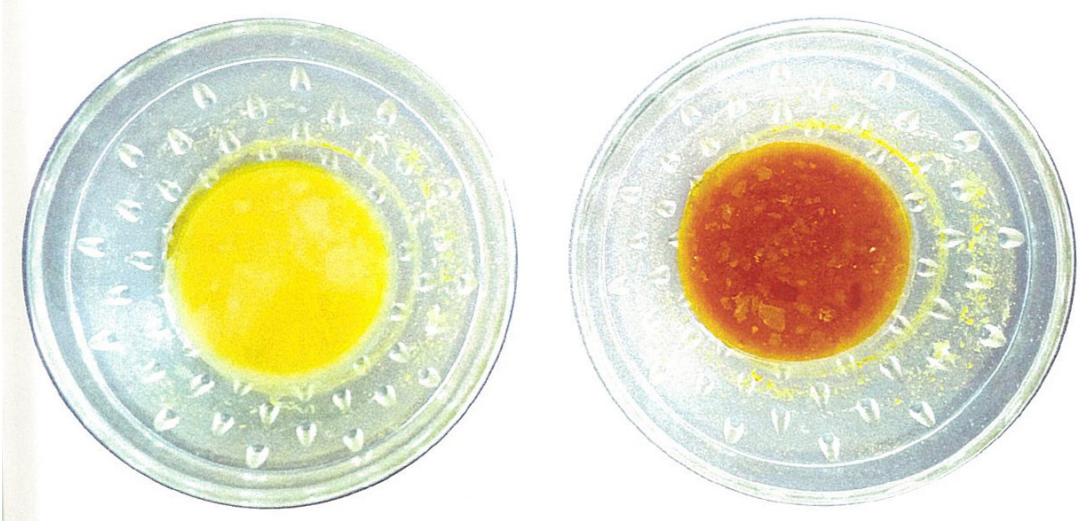
Figure No. 6

FINGER PRINT TEST



WATER FLOATING TEST

COLOUR CHANGING TEST



PHYSICOCHEMICAL ANALYSIS

PHYSICAL CHARACTERIZATION

The following characters have been noted in *VC*

Table no-6

Sl.No	Parameters	Result
1	Colour in day light	White
2	Smell	No significant
3	Taste	Tasteless
4	Appearance	Powder
5	Touch	Nice

INTERPRETATION:

The organoleptic characters of the drug *VENKARA CHUNNAM* showed that the color of the chunnam is white in colour since prepared from dry herbs and minerals, tasteless which might be responsible for the activity mentioned earlier and on sight they are nice powder.

Table no- 7 physicochemical Analysis

S.NO	Parameter	Result
1	Loss on drying at 70ºC	8.10±0.240
2	Acid insoluble ash	0.85±0.011
3	Water soluble ash	7.65±0.011
4	PH	9.540

[Values are mean of three determinations ±SEM]

INTERPRETATION:

Acid insoluble ash:

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. It is 0.85% for the drug.

Water soluble ash:

Water-soluble ash is the part of the total ash content, which is soluble in water. It is 7.65% for the drug

Loss on drying:

1. The total of volatile content and moisture present in the drug was established in loss on drying.
2. Low Moisture content of the drug reveals the stability and its shelf-life.
3. High moisture content can adversely affect the active ingredient of the drug.
4. Thus low moisture content could get maximum stability and better shelf life

MICROBIAL LIMIT TESTS

Table 8 : Results of Microbial Contamination Test

S.No.	Test Particulars	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	Total Viable Aerobic Bacterial Count	$5x10^3$	$1x10^5$
2.	Total Viable Fungal Count	$2x10^3$	$1x10^3$

Table 9 Results of Specific Pathogens Test

S.No.	Test for Specified Pathogens	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	*Salmonella* sp.	No growth	-
2.	*Staphylococcus aureus*	No growth	-
3.	*Escherichia coli*	No growth	-
4.	*Pseudomonas aeruginosa*	No growth	-

The Results of the microbiological analysis for microbial contamination of the drug venkara chunnam was given in Table 8. The total viable aerobic bacterial count was observed on Nutrient agar plate was 5 x $10^3$and the fungal count on SDA agar plates was 2 x10^3CFU/ g. This results were found to comply with the specification limit for total bacterial count i.e. NMT 1× 10^5 CFU/ml and not comply with the specification limit for total fungal count i.e. NMT 1× 10^3 CFU/ml (Protocol for testing Ayurveda, Siddha and Unani medicines).

The analytical screening of sample showed in Table 9 that the product is free from specific pathogen like *Escherichia coli*, *Salmonella, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Microbial contamination usually occurs because of improper drying or storage of the plant material which eventually results in degradation of the plant constituents. Microbial contamination can also render plant material toxic, either by transforming the chemicals in the plant material or through the production of toxic compounds by the microbes. Therefore, microbial quality tests should be applied to starting plant materials, intermediate and finished products where necessary. During the quality analysis, precautions must be taken to ensure that conditions do not adversely affect any microorganisms that are to be measured.

Thus, the present study proves that Venkara Chunnam is free from microbial contamination and also highlighted the safety of the same. The information obtained from microbial screening tests will be use full in finding out the quality of the drug

From these results, it is accomplished that this study would lead to the establishment of several important compounds that have to be used to formulate new, different and more potent antimicrobial drugs of natural origin. However, further studies are required to screen the biologically active compounds and to evaluate the efficiency of this compound against pathogenic microorganisms associated with various human diseases.

BIO-CHEMICAL ANALYSIS OF *"VENKARA CHUNNAM"*

Table No: 10 Preliminary tests for basic and acidic radical:

S.NO	EXPERIMENT	INFERENCE
1.	Test For Calcium	Present
2.	Test For Sulphate	Present
3.	Test For Chloride	Present
4.	Test For Carbonate	Absent
5.	Test For Starch	Absent
6.	Test For Iron Ferric	Absent
7.	Test For Iron Ferrous	Present
8.	Test For Phosphate	Present
9.	Test For Albumin	Absent
10.	Test For Tannic Acid	Absent
11.	Test For Unsaturation	Present
12.	Test For The Reducing Sugar	Absent
13.	Test For Amino Acid	Absent
14.	Test For Zinc	Absent

INTERPRETATION:

Calcium:

- Calcium ions are necessary for the maintenance and regulation of acid-base balance and water balance in the body.
- Calcium interacts with troponin C to trigger the muscle contraction.
- Several reactions in the cascade of blood clotting process are dependent on ca^{2+}(factor IV).
- Calcium influences the membrane structure and transport of water and several ions across it.

Sulphate:

- Nutritionally essential element.
- Sulphate has anti bacterial activity and it is one of the macronutrient of cells.

- It inhibits growth of yeasts and moulds in low pH and inhibits growth of enterobacteriae and other gram negative bacteria in high pH.
- Sulphate important role for the anti-microbial activity.
- It is needed to start the cascade of digestive enzymes released from the pancreas. Without proteases, lipases and amylases, food is not digested efficiently.

Chloride

- Chloride forms the chief anion of the extracellular fluid and exists along with sodium mostly.
- Regulates acid base balance.
- Formation of HCl in gastric juice.
- Help to preserve normal neuromuscular irritability by maintaining a state of eqilibrium, on account of their relative proportion in ECF and ICF.

Iron:

- Iron is associated with effective immune competence of the body.
- Iron is important for the formation of Hemoglobin, Myoglobin and other substances like cytochrome, cytochrome oxidase, peroxidase and catalase.

Phosphate:

- Phosphate is also required for the development of bones and teeth.
- Adenosine triphosphate, main source of energy in cells, must be bound to the Phosphate ion in order to be biologically active.
- Phosphate is necessary for proper neuromuscular function.

Unsaturated Compound :

- Monounsaturated and polyunsaturated fats can replace saturated fat in the diet, trans unsaturated fats should not.
- Replacing saturated fats with unsaturated fats helps to lower levels of total cholesterol and LDL cholesterol in the blood.

PHYTO-CHEMICAL STUDY OF VENGARA CHUNNAM

This experimental study was taken up to qualitative analysis of Phyto-chemicals in the drug of ***Venkara Chunnam*** using various test and the results are exhibited in Table No..11

Table No 11: Incidence of various phyto-chemicals in *Venkara Chunnam*

S.No.	Name of Tests Conducted	Result Observed
Observation of Alkaloids		
1.	Mayer's Test	Negative
2.	Dragendroff's Test	Negative
3.	Hager's Test	Positive
Observation of Carbohydrates and Glycosides		
4.	Molisch Test	Positive
5.	Legal's Test	Negative
6.	Borntrager's Test for anthraquinones	Negative
Observation of Phytosterols		
7.	Liebermann – Burchard Test	Negative
8.	Salkowski Test	Negative
Observation of Flavanoids		
9.	Shinoda Test (Magnesium turnings & Hydrochloric acid)	Negative
10.	Fluorescence Test	Negative
Observation of Tannins		
11.	Ferric chloride test	Negative
12.	Potassium dichromate test	Negative
13.	Lead acetate test	Positive
14.	Millon's test	Negative
15.	Biuret test	Positive
16.	Ninhydrin test	Negative
Observation of fixed oils and fats		
17.	Spot test	Negative
18.	Saponification test	Negative
Observation of Lignin		
19.	Phloroglucinol test	Negative
Observation of Saponins		
20.	Frothing test	Negative

Note: *Positive indicates the presence of Phytochemical;*
Negative indicates the absence of Phytochemical

RESULTS:

Result shows the presence of Alkaloids, carbohydrates and glycosides, tannins, and absence of phytosterols, flavonoids, fixed oil, fat, proteins, saponins and lignin.

INTERPRETATION:

Alkaloids:

Alkaloids have a wide range of pharmacological activities including analgesic, antimicrobial activity.

Glycosides:

Serves as reserve food material, means for removal of toxic substances inhibit disease producing microorganisms.

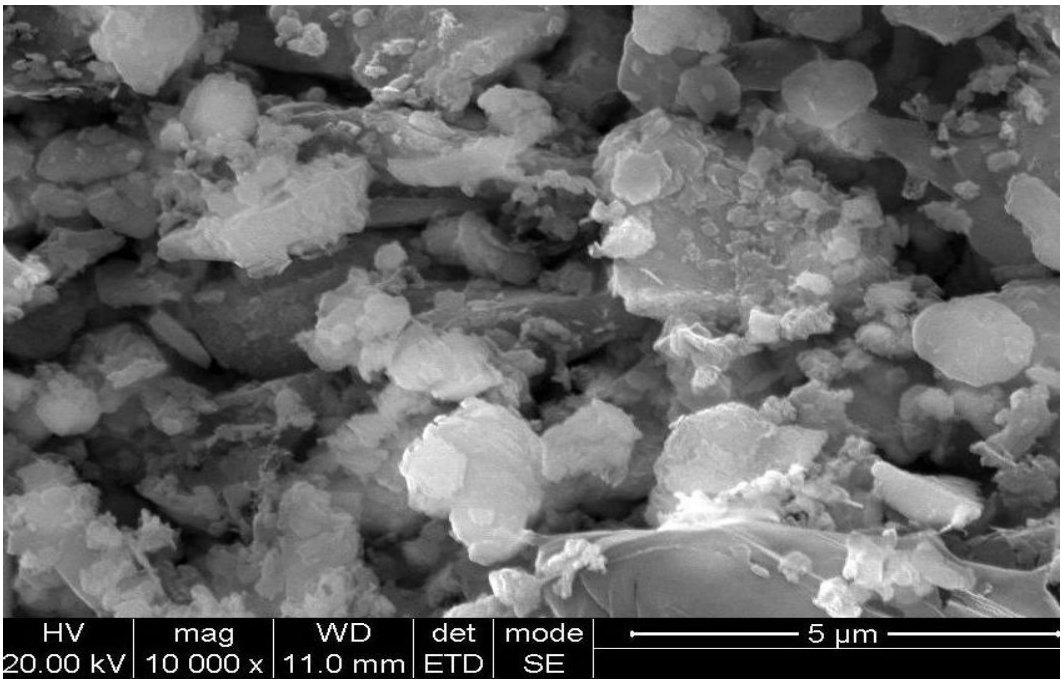
Tannins:

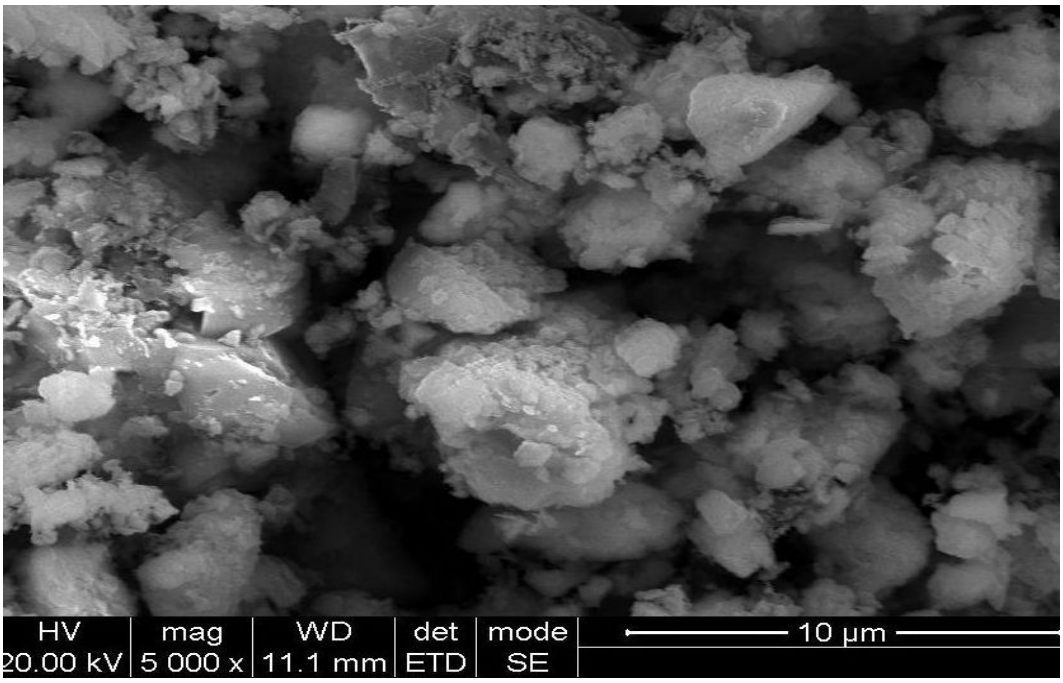
Tannins are considered antioxidants property, creating an insulating and protective layer that soothes irritation and pain of the skin.

INSTRUMENTAL ANALYSIS

SEM RESULT

Figure no:7





INTERPRETATION:

The morphology of the *VENKARA CHUNNAM* sample can be determined by Environmental SEM (FEI Quanta). A representative portion of each sample must be sprinkled onto a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination. We have observed from SEM photographs that particles are spherical in shapes and sizes are in the range from 10 micron to 5 micron. Although the particle sizes of different batches showed similarity, it seems that these particles are aggregates of much smaller particles. When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gives these particles a tendency to aggregate together to form larger particles. *VENKARA CHUNNAM* exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation.

FOURIER TRANSFORM – INFRA RED SPECTRO SCOPY (FTIR)

Fourier Transform Infra-Red spectroscopy (FTIR) analysis results in structure spectra that provide information about the functional group and molecular structure of a material IR relates with the sample and the bonds among atoms in the molecule stretch and bend absorbing infrared energy and creating the infrared spectrum. It is of two kinds of bending and stretching.

FTIR is very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation so confirming the presence of active molecule responsible for the therapeutic activity of siddha drugs. The result of following tables shows the presence of functional groups and inorganic compounds of venkara chunnam.

FTIR RESULTS OF *VC*:

Figure No: 8 FTIR Result Graph

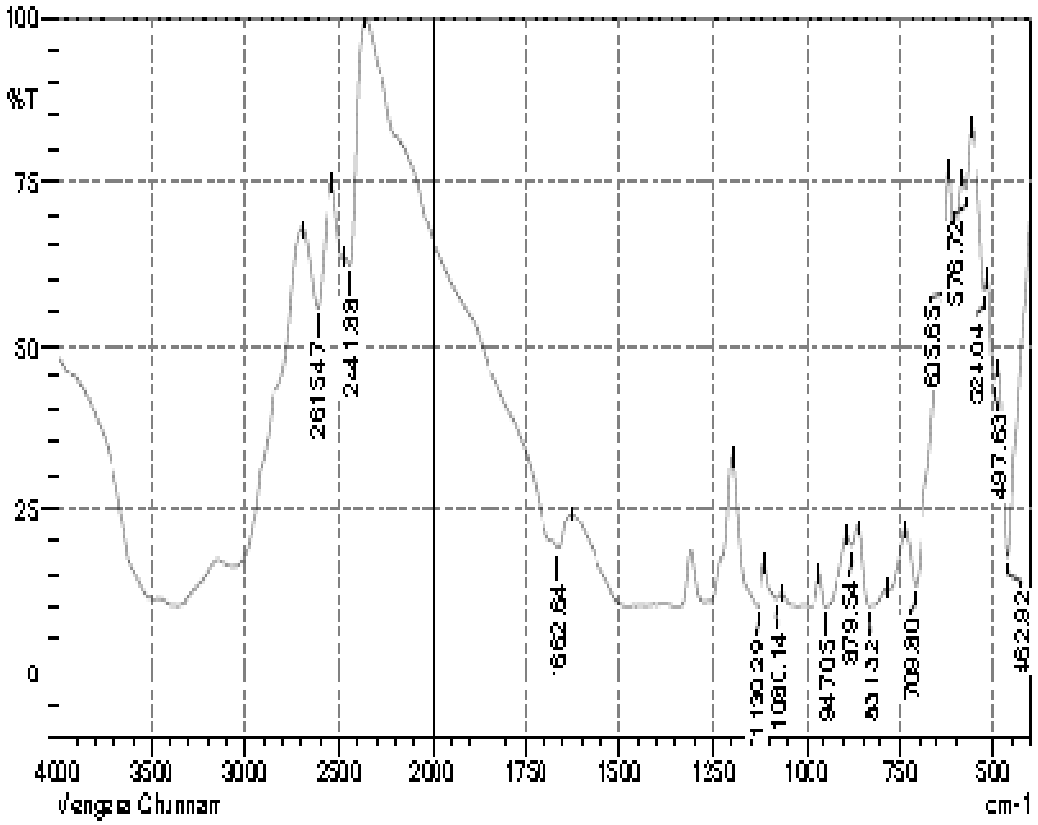


Table no - 12 Showing represented functional groups

Absorption peak cm^{-1}	Stretch	Functional group
2615.47	O-H – Stretch	Carboxylic acid
2441.88	O-H – Stretch	Carboxylic acid
1662.64	-C=C - Stretch	Alkene
1130.29	C-O- Stretch	Alchols, Esters, Ethers, Carboxylic acid
1080.14	C-N- Stretch	Aliphatic amines
947.05	N-H-Stretch	Primary and Secondary Amines.
879.54	N-H-Stretch	Primary and Secondary Amines.
831.32	N-H-Stretch	Primary and Secondary Amines.
709.80	N-H-Stretch	Primary and Secondary Amines.
605.65	C-Br- Stretch	Alkyl halides
576.72	C-Br- Stretch	Alkyl halides
524.64	C-Br- Stretch	Alkyl halides
497.63	C-C Stretch	Cyloalkane
462.92	C-C Stretch	Cyloalkane

INTERPRETATION:

In FTIR the wave numbers between $4000cm^{-1}$-$400cm^{-1}$. 4000 cm^{-1}-1500 cm^{-1} is known as functional group area.1500 cm^{-}1-$600cm^{-1}$ is known as finger print area. The identity of FTIR of two compounds is much more characteristic than the comparisons of their many physical properties. The corresponding absorption frequency by FTIR shows the presence of Carboxylic acid, alkenes, alcoholic groups, aliphatic amines, primary and secondary amines, alkyl halides, cyloalkane, esters and ethers.

Alcoholic groups:

- Alcoholic group of substances act as antimicrobial and antiseptic agents.

Carboxylic acid:

- This group of substance has antimicrobial, anticoagulant activity.

Amines groups:

- Amines groups act as neurotransmitters.
- It is involved in protein synthesis.
- This group of substances has analgesic activity.

Alkenes groups:

- Alkenes have little biological activity.
- It is predominate in plants. They protect against bacteria and fungi.

Alkyl halides:

- These are group of compounds derived from alkanes containing one or more halogens.Some are used as anesthetics and antiseptic agents. Some of them are used in medicine for the elimination of hook worms.

Esters:

- This group is produced a diuresis and natriuresis. So result of a direct tubular action together with a pressure diuresis

Ethers:

- Ether which increases the flow of urine
- It is a best diuretic action.
- A diffusible stimulant and antispasmodic used chiefly in colic.

ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy):

The drug *VC* sample was analysed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively.

The result of ICP-OES is given on the Venkara Chunnam

Table No: 13.

Sample ID	Elements Symbol	Wavelength(nm)	Concentration (A)P
1.	Al	396.152	BDL
2.	As	188.979	BDL
3	B	249.677	405.214mg/L
4.	Ca	315.807	2.170 mg/L
5.	Cd	228.802	BDL
6.	Cu	327.393	BDL
7.	Fe	238.204	0.016mg/L
8.	Hg	253.652	BDL
9.	K	766.491	03.004 mg/L
10.	Mg	285.213	01.004 mg/L
11.	Na	589.592	701.100 mg/L
12.	Ni	231.604	BDL
13.	Pb	220.353	BDL
14.	P	213.617	106.351 mg/L

BDL:Below Detectable Limit (Normal – 1 ppm)

1% = 10000ppm,

1ppm = 1/1000000 or 1ppm = 0.0001%

The toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic (As)	10ppm
Mercury (Hg)	1ppm
Lead (Pb)	10ppm
Cadmium (Cd)	0.3ppm

Plants are the rich source of elements. These elements have vital function. They were significant role against a variety of diseases. Hence they were used for several health problems.

The *VC* showed presence of K, Mg, P, Na, B, Fe, Ca. In *VENKARA CHUNNAM,* the heavy metals like As, Hg, Cd, Pb, Cu, Ni, Al were below detectable level. The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

Interpretation:

Potassium:

In the presence of Sodium and Potassium regulate the acid-base balance of the body fluids.They regulate the water balance of the body fluids.They help to preserve the neuromuscular irritability by maintaining a state of equilibrium on account of their relative proportion in the Extra cellular fluid and Intra cellular fluid.Potassiumis an electrolyte critical in maintaining proper acid-alkaline balance. Potassium is highly present inintra cellular fluid(ICF), while its partner, sodium, is highly present in extra cellular fluid(ECF).It is their perfect ratio that maintains fluid and electrolyte balance. Potassium dilates the arteries and relaxes the smooth muscles and increases the blood flow (F.J. Haddy, 2012).

Magnesium:

Magnesium inhibits the formation of calcium-oxalate crystals in the urine. It also inhibits the stone formation by inhibition of growth of crystals as well as aggregation. Inhibition of crystal attachment of calcium oxalate appears to require supra-physiologic concentrations. Magnesium reduces the formation of stones by reducing the calcium oxalate in the blood that makes up the stones.

Phosphorus:

Phosphorus is an important constituent of phosphate buffers in the blood and urine. It is required for the formation of certain physiologically important phosphorus containing compounds like phospholipids, coenzymes and enzymes of intermediary metabolism.

Sodium:

Sodium regulates the acid base balance of the body fluids. Sodium is required for the maintenance of the osmotic pressure. It is necessary for the normal

muscle irritability and permeability of cells. Sodium is involved in the intestinal absorption of glucose and amino acids.

Boran

Boricacid is anti-microbial and several natural boran containing organic anti-biotics. Boran supplementation markedly reduced urinary calcium excreation and elevated the serum concentration of 17 beta estradiol and testosterone.

Ferrous iron

Iron supplementation resulted in a significant decreases eosinophilia, while systemic iron injections lead to a significant suppression of both allergen-induced eosinophilia and hyperactivity compared to placebo

Calcium:

Calcium is an improntant comonent of cell memberane it control permiability and electrical properties of the cell membrane. Calcium necessary for the maintenance and regulation of acid - base balance and water balance in the body. Calcium helps to prevent kidney stone formulation.

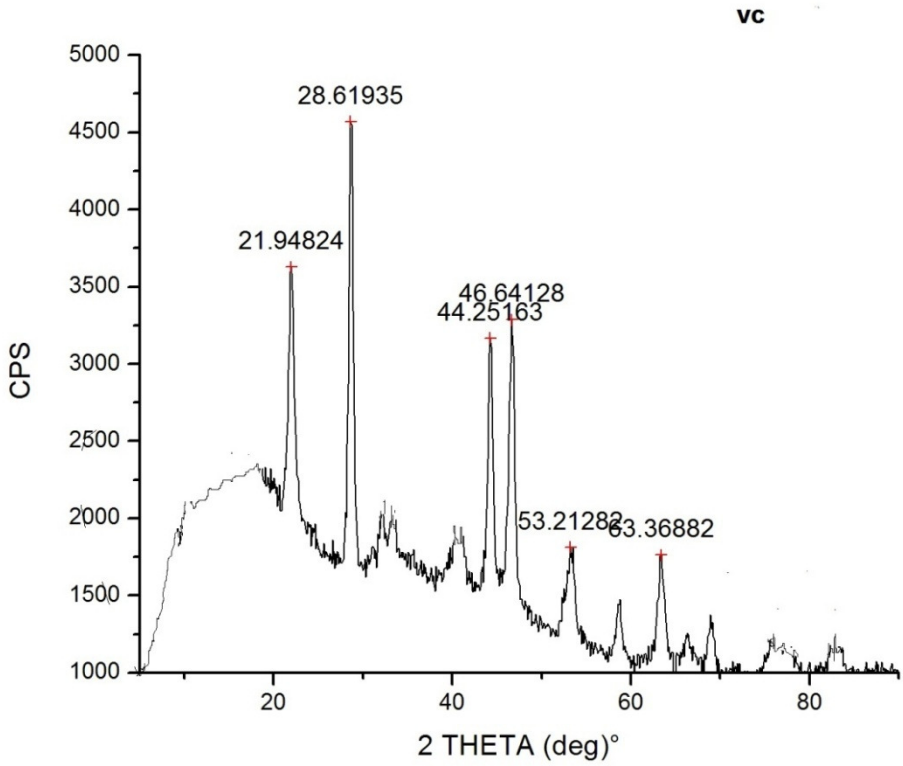
XRD (X-RAY POWDER DIFFRACTION)

The drug *VC* sample was analysed by the X-ray powder diffraction (XRD) to checking the quality control of above mineral medicines

The result of XRD is given on the Venkara Chunnam

Figure no. 9

XRD Image of *VENKARA CHUNNAM*



Interpretation:

This XRD finger print shows both the similarities and difference of the sample successfully and is a valuable primary tool for checking the quality control of above mineral medicines. Modern techniques are necessary to standardize and bring out high quality herbal product owing to their complex nature. The different peak shows the presence of minerals in the sample.

TOXICITY STUDIES

EVALUATION OF ACUTE TOXICITY STUDY ON *VENKARA CHUNNAM*

Effect of Acute Toxicity Study (14 Days) of *VC*

Table no –14 Physical and behavioral examinations.

Group No.	Dose(mg/kg)	Observation sign	No. of animal affected.
Group-I	5mg/kg	Normal	0 of 3
Group- II	50mg/kg	Normal	0 of 3
Group-III	300mg/kg	Normal	0 of 3
Group-IV	1000mg/kg	Normal	0 of 3
Group-V	2000mg/kg	Normal	0 of 3

Table no-15 Home cage activity

Functional and Behavioural observation	Observation	5mg/kg Group (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Table no-16 Hand held observation

Functional and Behavioral observation	Observation	Control	5 mg/ kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Table no-17 Mortality

Group no	Dose no(mg/kg)	Mortality
Group-I	5(mg/kg)	0 of 3
Group-II	50(mg/kg)	0 of 3
Group-III	300(mg/kg)	0 of 3
Group-IV	1000(mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

RESULT:

From acute toxicity study it was observed that the administration of *VC* at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of *VC* is 2000 mg/kg.

DISCUSSION

VENKARA CHUNNAM was administered single time at the dose of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioral signs of any toxicity due to administration of ***VC*** at the doses of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominal tone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups. Food consumption of all treated animals was found normal as compared to normal group.

Body weight at weekly interval was measured to find out the effect of ***VENKARA CHUNNAM*** on the growth rate. Body weight change in drug treated animals was found normal.

INTERPRETATION:

VENKARA CHUNNAM was administered single time at the dose of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioral signs of any toxicity due to administration of ***VENKARA CHUNNAM*** at the doses of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominal tone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups. Food consumption of all treated animals was found normal as compared to normal group.

Body weight at weekly interval was measured to find out the effect of ***VENKARA CHUNNAM*** on the growth rate. Body weight change in drug treated animals was found normal.

SUB-ACUTE TOXICITY STUDY IN WISTAR RATS TO EVALUATE TOXICITY PROFILE OF *VC*

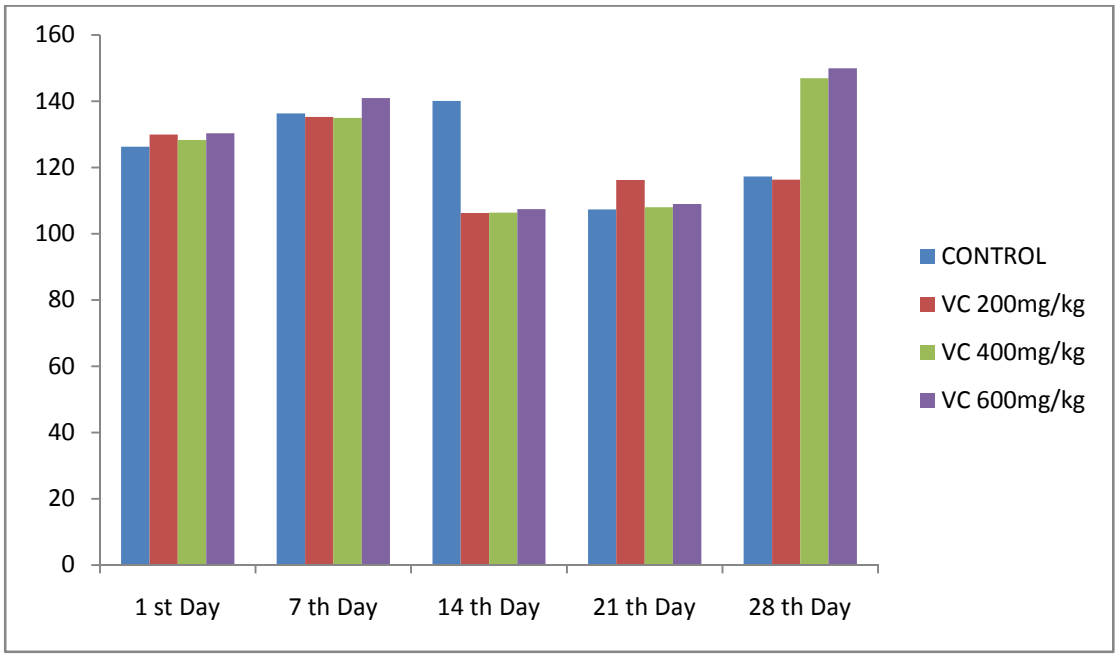
Table: 18 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *VC ON* BODY WEIGHT IN GRAM

GROUP	CONTROL	VC (200mg/kg)	VC (400mg/kg)	VC (600mg/kg)
1st DAY	126.3±1.430	130±1.943	128.3±2.631	130.3±2.63
7th DAY	136.3±1.430	135.3±1.443	135±2.513	141±2.510
14th DAY	140.1±1.404	106.3±1.520	106.4±2.412	107.4±2.412
21st DAY	107.3±2.520	116.2±1.901	108±1.531	109±1.530
28th DAY	117.3±1.441	116.3±1.602	147±2.4405	150±2.440

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's (n=6); ^{ns} $p>0.05$, $*p<0.05$, $**p<0.01$, $***p<0.001$, calculated by comparing treated groups with control group.

BODY WEIGHT

Figure 10:



EFFECT OF SUBACUTE DOSE (28 DAYS) OF *VENKARA CHUNNAM*

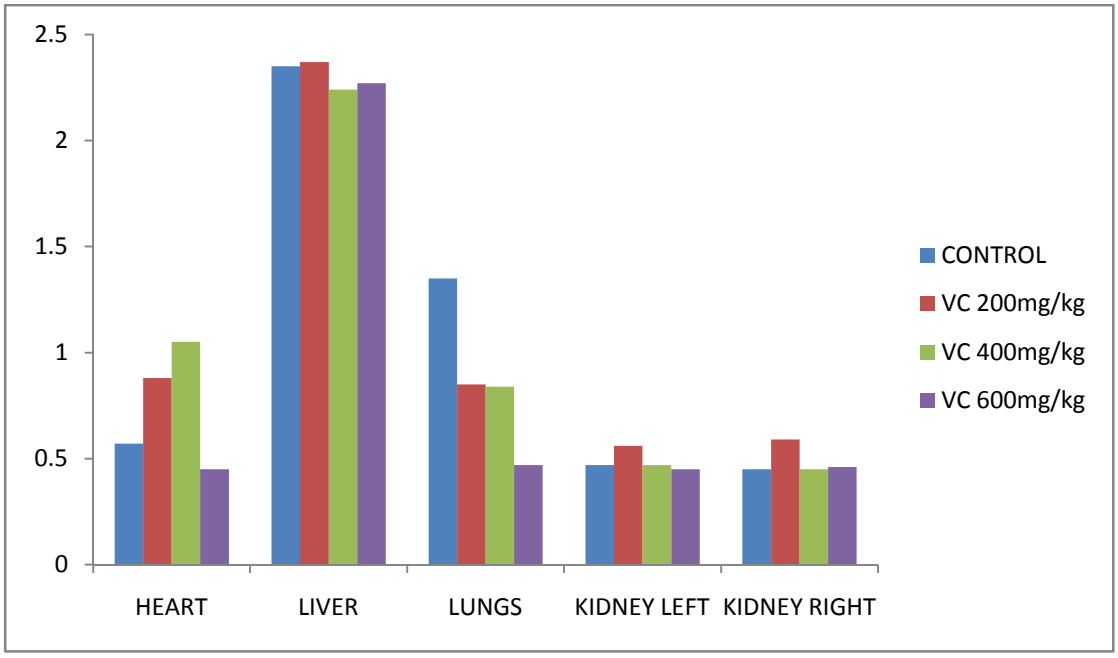
Table: 19 *VC* ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM

GROUP		CONTROL	VC (200mg/kg)	VC (400mg/kg)	VC (600mg/kg)
HEART		0.57±0.42	0.88±0.44	1.05±0.51	0.45±0.42
LIVER		2.35± 0.63	2.37±0.63	2.24±0.41	2.27± 0.63
LUNGS		1.35±0.50	0.85±0.54	0.84±0.64	0.47±0.50
KIDNEY	Left	0.47±0.42	0.56±0.43	0.47±0.42	0.45±0.42
	Right	0.45±0.424	0.59±0.42	0.45±0.424	0.46±0.424

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's (n=6); ^{ns} p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Figure 11:

ORGAN WEIGHT



EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *VENKARA CHUNNAM ON* HAEMATOLOGICAL PARAMETERS

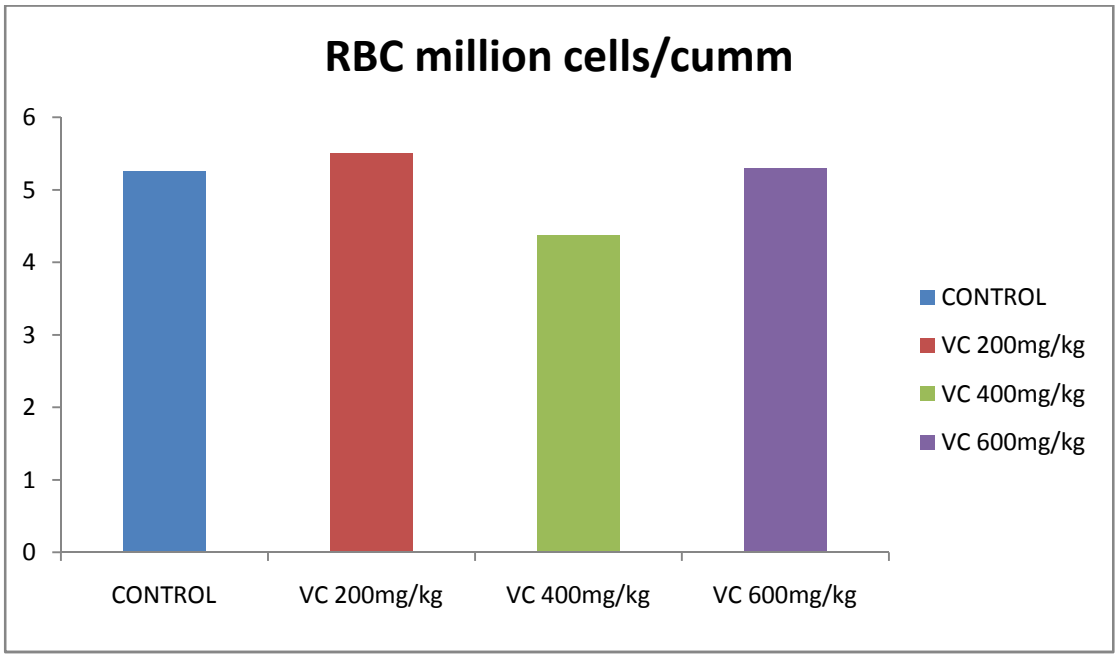
Table no 20

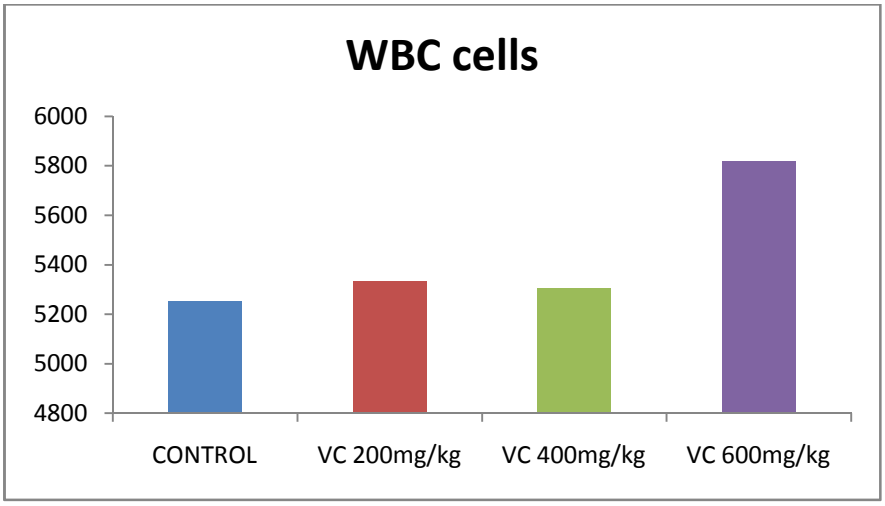
Drug treatment	RBC million cells /cu mm	WBC cells/cu mm	Haemoglobin gm %	Differential count %			
				Neutrophils	Eosinophil's	Monocyte	Lymphocytes
Control	5.25±0.80	5252.45±23.72	12.44±0.85	31.31±1.60	3.57±0.51	0.69±0.55	23.17±3.72
LOW	5.51±0.60	5334.08±23.62	13.24±0.83	25.58±1.81	2.14±0.54	0616±0.70	23.26±3.91
MID	4.37±0.61	5304.29±32.75	14.15±1.43	30.36±2.62	2.48±0.52	0.66±0.80	23.17±3.72
HIGH	5.30±0.61	5818.29±32.75	16.15±1.43	28.36±2.62	2.54±0.52	0.68±0.80	24.17±3.72

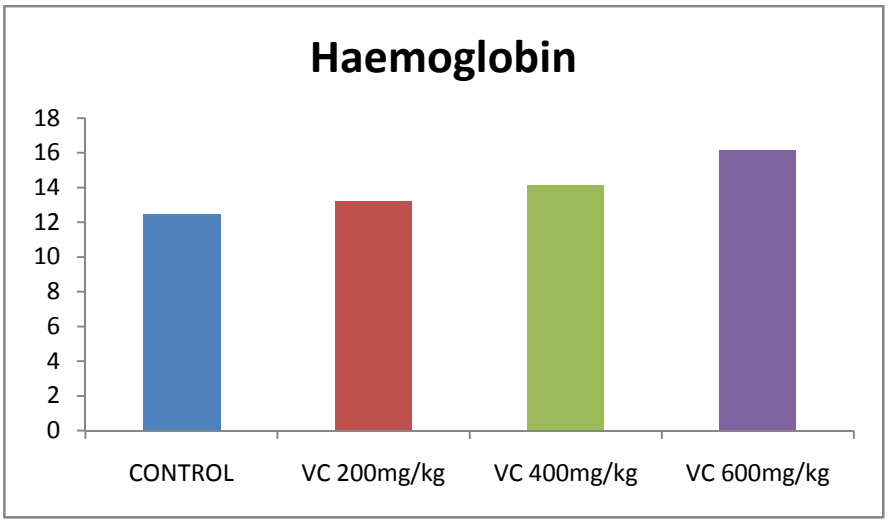
Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's (n=6); ^{ns} p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

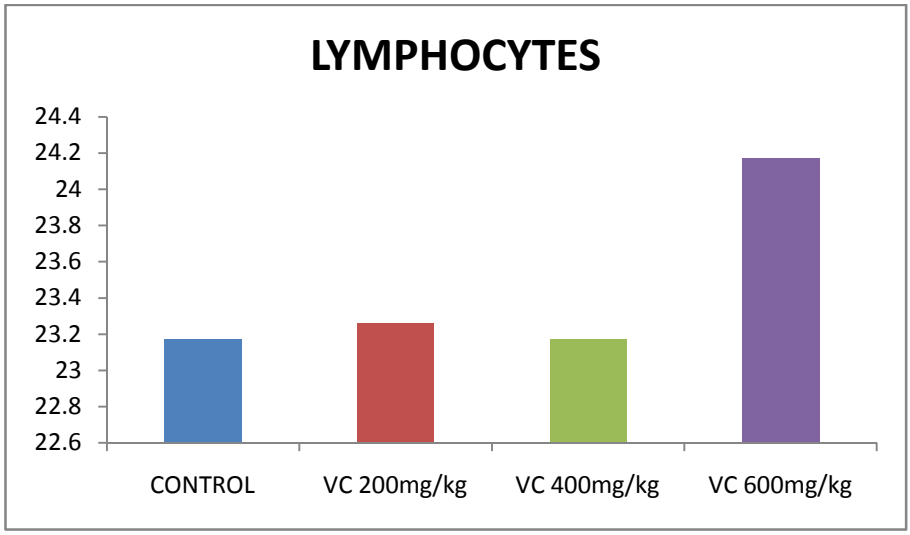
Effect of Sub acute dose (28 days) of VC on Hematological parameters

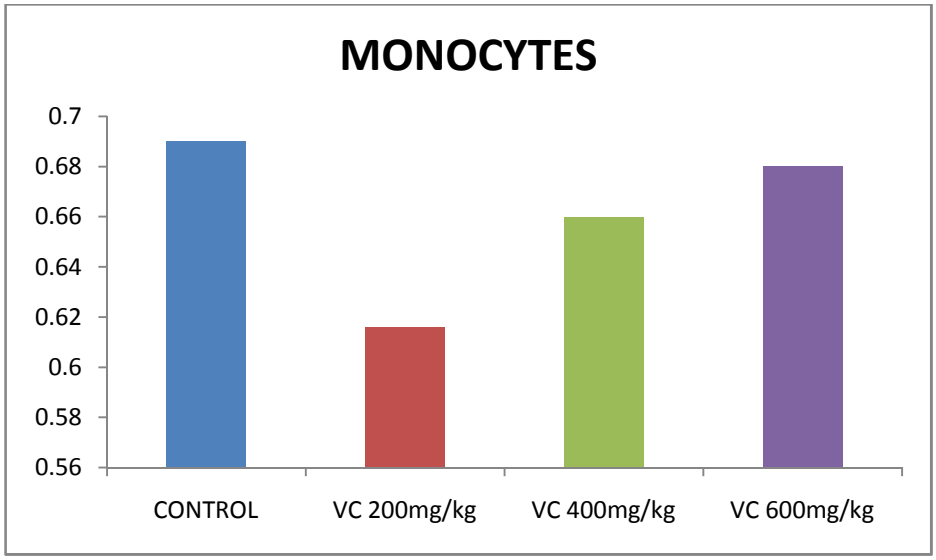
Figure 12:

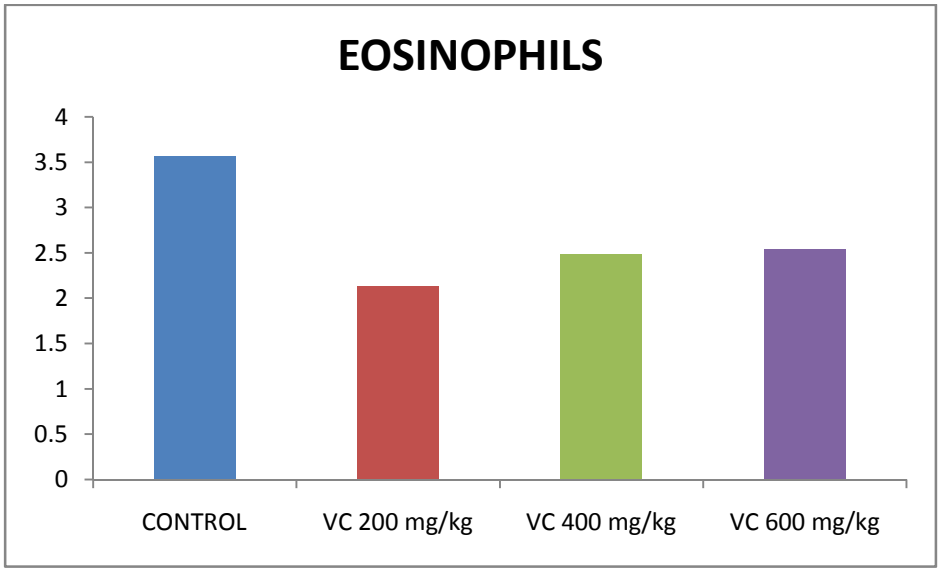


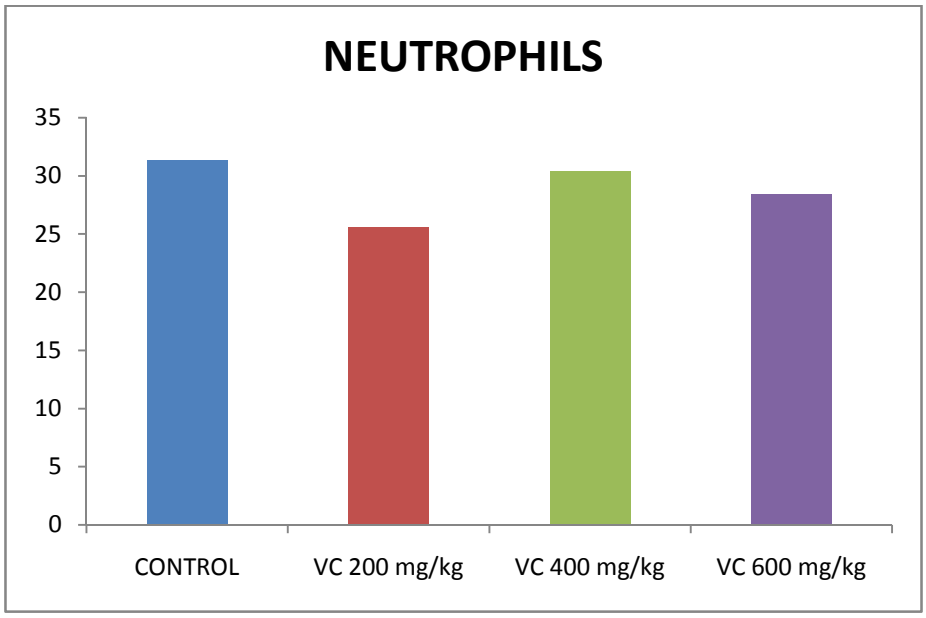

WBC cells
6000
5800
5600
5400
5200
5000
4800
CONTROL
VC 200mg/kg
VC 400mg/kg
VC 600mg/kg


Haemoglobin
18
16
14
12
10
8
6
4
2
0
CONTROL
VC 200mg/kg
VC 400mg/kg
VC 600mg/kg


LYMPHOCYTES
24.4
24.2
24
23.8
23.6
23.4
23.2
23
22.8
22.6
CONTROL
VC 200mg/kg
VC 400mg/kg
VC 600mg/kg


MONOCYTES
0.7
0.68
0.66
0.64
0.62
0.6
0.58
0.56
CONTROL
VC 200mg/kg
VC 400mg/kg
VC 600mg/kg


EOSINOPHILS
4
3.5
3
2.5
2
1.5
1
0.5
0
CONTROL
VC 200 mg/kg
VC 400 mg/kg
VC 600 mg/kg

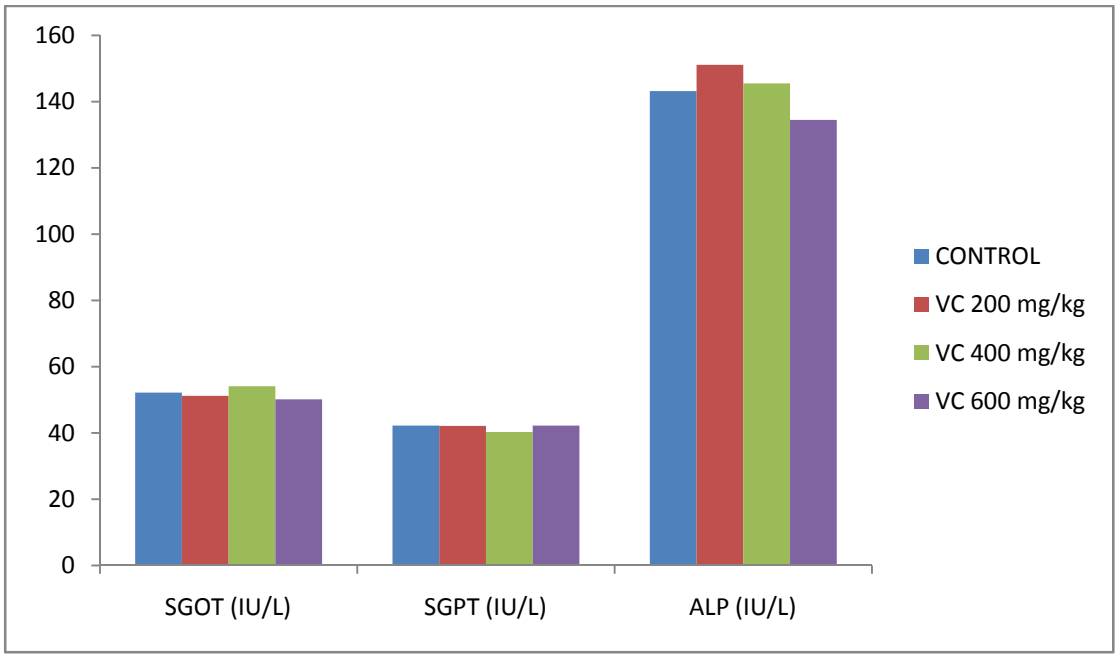

NEUTROPHILS
35
30
25
20
15
10
5
0
CONTROL
VC 200 mg/kg
VC 400 mg/kg
VC 600 mg/kg

EFFECT OF SUB ACUTE DOSE (28 DAYS) OF VC ON HEMATOLOGICAL PARAMETERS

Table: 21 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *VC* ON BIOCHEMICAL PARAMETERS

GROUP	CONTROL	VC (200mg/kg)	VC (400mg/kg)	VC (600mg/kg)
SGOT (units/min/liter/mg protein)	**52.18±4.71**	**51.17±4.41**	**54.15±2.61**	**50.15±2.61**
SGPT (units/min/liter/mg protein)	**42.18±3.42**	**42.17±3.62**	**40.25±4.84**	**42.25±4.84**
ALP (units/min/liter/mg protein)	**143.16±11.72**	**151.15±12.82**	**145.49±4.54**	**134.49±4.54**

Figure 13: BIO CHEMICAL PARAMETERS



EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *VENKARA CHUNNAM*

BIOCHEMICAL PARAMETERS

Table 22

GROUP	CONTROL	VC (200mg/kg)	VC (400mg/kg)	VC (600mg/kg)
TOTAL BILIRUBIN (mg/dl)	0.8±057	0.68±0.27	0.88±0.36	0.504±0.199

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's (n=6); ^{ns} $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, calculated by comparing treated groups with control group.

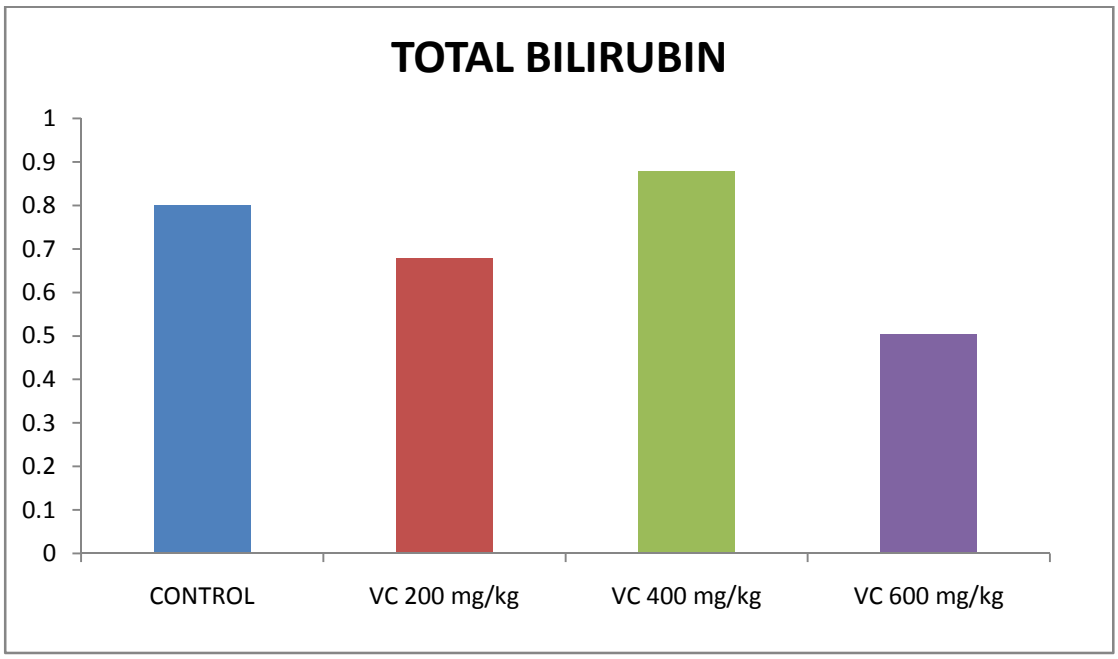
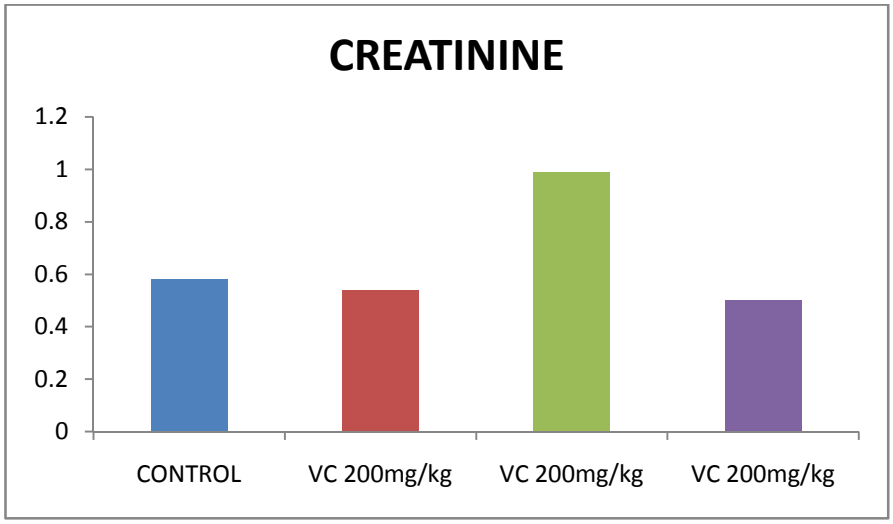


Table: 23 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *VENKARA CHUNNAM ON* BIOCHEMICAL PARAMETERS

GROUP	CONTROL	VC (200mg/kg)	VC (400mg/kg)	VC (600mg/kg)
CREATININE (mg/dl)	**0.58±0.43**	**0.54±0.44**	**0.99±0.44**	**0.50±0.44**
UREA (mg/dl)	**45.39±3.40**	**50.57±2.82**	**53.16±2.62**	**40.16±2.62**

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's (n=6); ^{ns} p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Figure 14: BIOCHEMICAL PARAMETERS OF UREA AND CREATININE



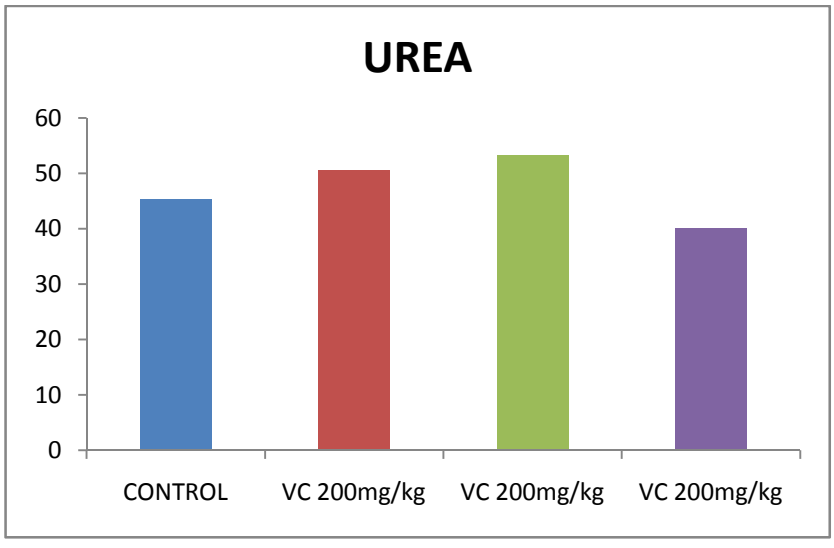


Table: 24 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *VENKARA CHUNNAM* ON FOOD INTAKE IN GRAM

GROUP	CONTROL	VC (200mg/kg)	VC (400mg/kg)	VC (600mg/kg)
1st DAY	16.37±3.10	18.1-6±1.7	12.14±2.1	17.9±8.02
7th DAY	13.9±1=1.4	11.7±3.07	16.77±1.23	11.21±14.81
14th DAY	15.87±0.12	10.8±.68	14±16.36	19.76±9.381
21st DAY	15.91±2.8	15.4±8.8	15.92±9.83	19.21±8.42
28th DAY	13.14±1.78	18.2±11.0	10.4±9.30	10.4±7.97

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's (n=6); ^{ns} $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, calculated by comparing treated groups with control group

Figure 15: FOOD INTAKE

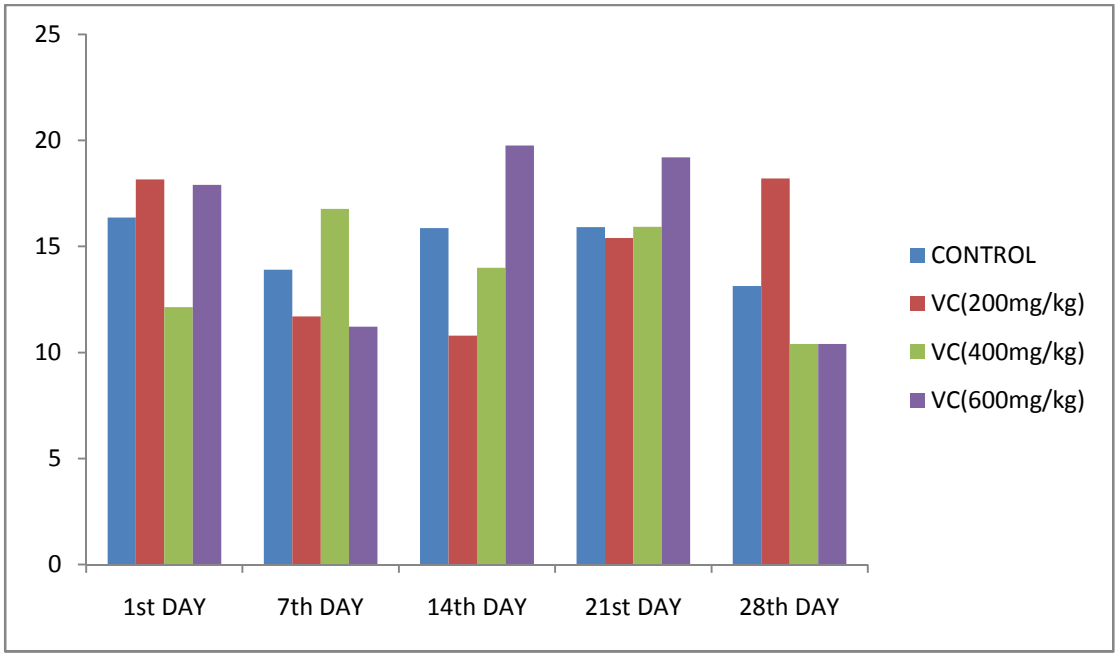


Table: 25 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *VENKARA CHUNNAM* ON WATER INTAKE IN ML

GROUP	CONTROL	VC (200mg/kg)	VC (400mg/kg)	VC (600mg/kg)
1st DAY	98.8±13.10	89.72±14.36	102.10±21.99	77.5±7.203
7th DAY	85.5±11.738	100.863±12.60	76.63±9.3	81.17±14.40
14th DAY	88.33±8.727	90.83±14.2812	80±13.2	89.1672±8.981
21st DAY	91.87±12.49	85±8.462	75.38±9.40	89.1717±8.92
28th DAY	82.10±11.30	88.48±11.04	80±8.1	70±7.53

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's (n=6); ^{ns} $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, calculated by comparing treated groups with control group

Figure 16: WATER INTAKE

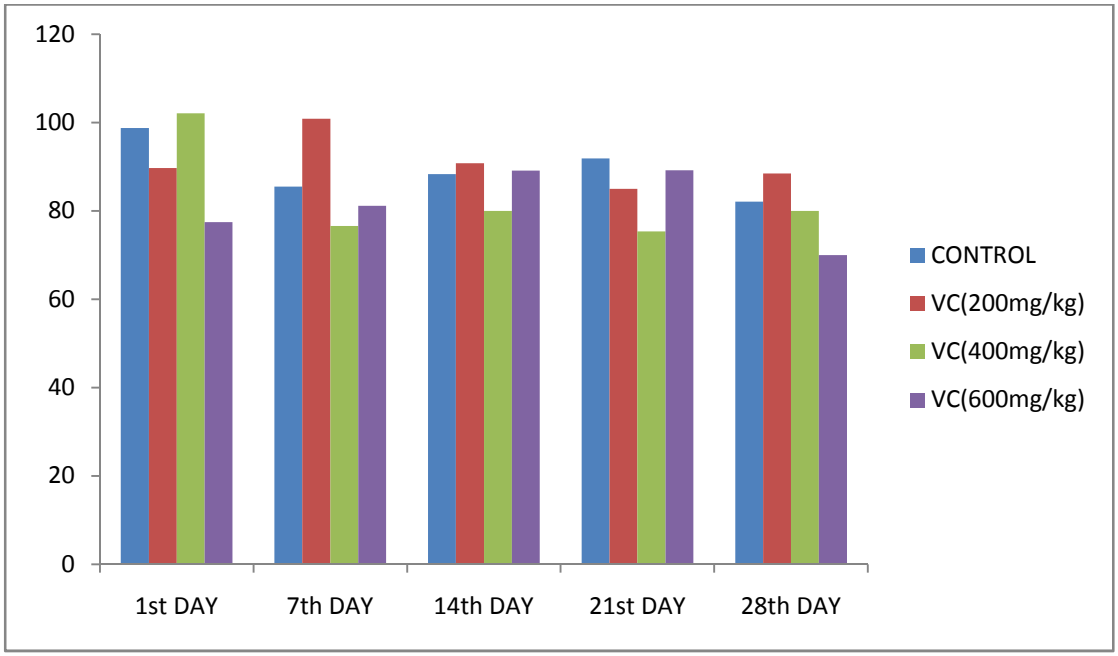
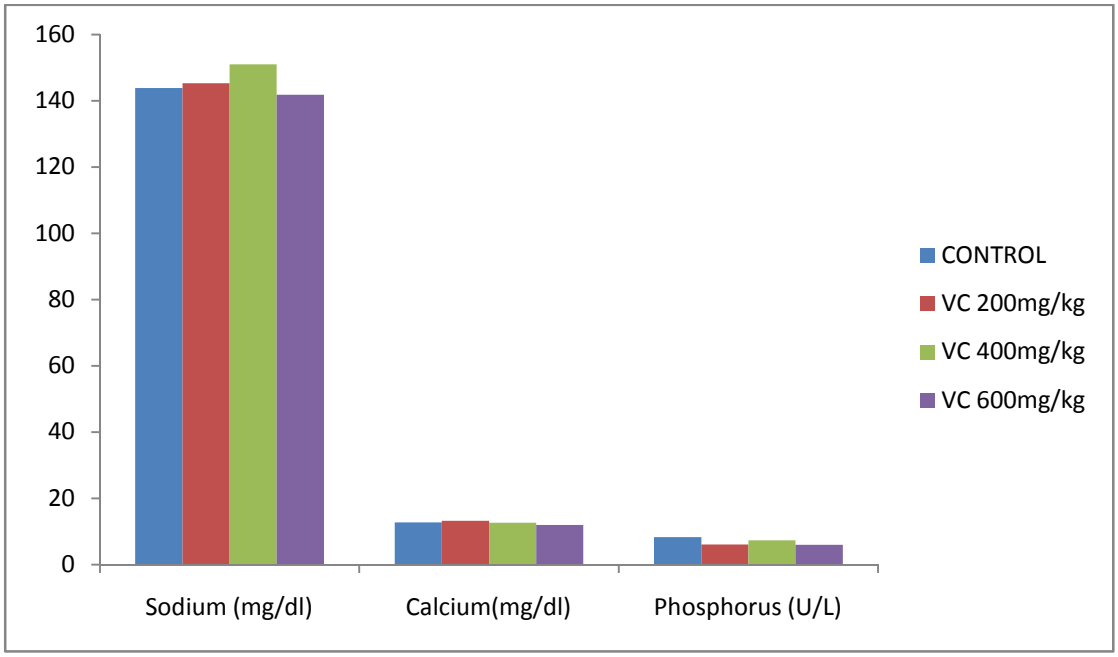


Table: 26 EFFECT OF SUB ACUTE DOSES (28 DAY) OF *VENKARA CHUNNAM* ON ELECTROLYTES:

GROUP	CONTROL	VC (200mg/kg)	VC (400mg/kg)	VC (600mg/kg)
Sodium (mg/dl)	143.910±0.5	145.30±0.2	151±0.71	141.80±0.0
Calcium(mg/dl)	12.80±0.19	13.20±0.73	12.7±0.9*	12.0±0.**
Phosphorus (U/L)	8.28±0.7	6.10±0.05^{ns}	7.30±0.9^{ns}	6.0±0.2*

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's (n=6); NS- non-significant, *p<0.05, **p<0.01, ***p<0.001,

Figure 17: ELECTROLYTES



6.0 RESULTS:

CLINICAL SIGNS:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

Mortality:

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

Body weight:

Results of body weight determination of animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Organ Weight:

Group Mean Relative Organ Weights (% of body weight) are recorded in Table No.19 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Hematological investigations:

The results of hematological investigations conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Biochemical Investigations:

Results of Biochemical investigations conducted on the day 29th and recorded in Table no 21, 22 revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

INTERPRETATION:

1) All the animals from control and all the treated dose groups up to 15ml/kg survived throughout the dosing period of 28 days.
2) No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.
3) Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.
4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days
5) Haematological analysis conducted at the end of the dosing period on day 29th, revealed no abnormalities attributable to the treatment.
6) Biochemical analysis conducted at the end of the dosing period on day 29^{th}, no abnormalities attributable to the treatment.
7) Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

PHARMACOLOGICAL STUDY

1. LITHOTRIPTIC ACTIVITY

Effect on urinary output in urolithiasis induced rats

Table No: 27

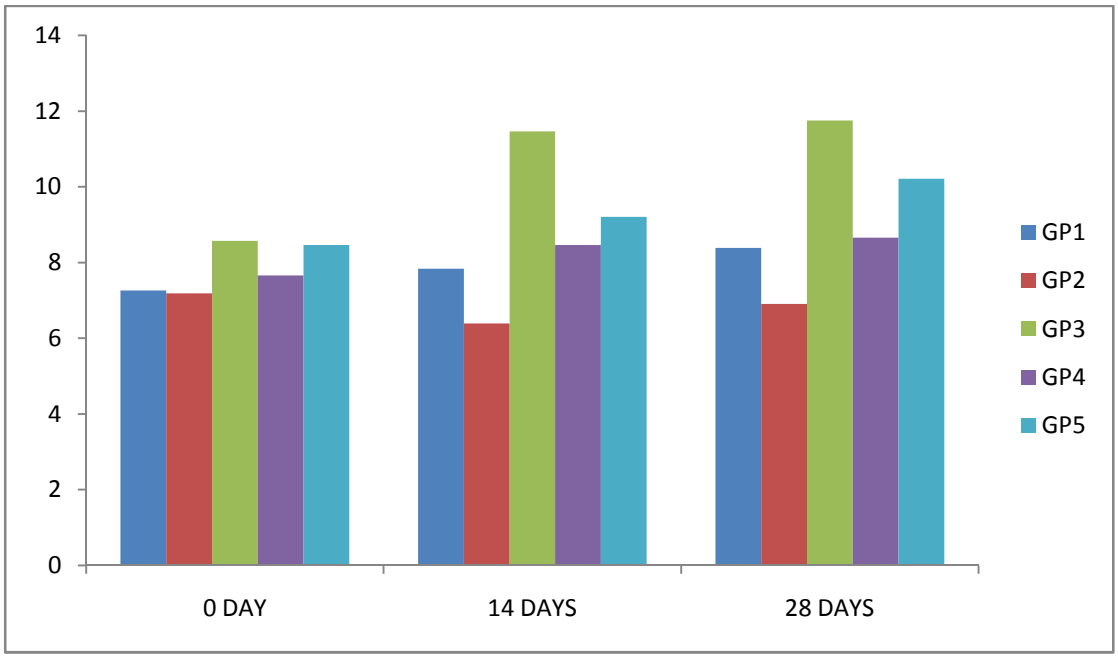
Days	GP1	GP2	GP3	GP4	GP5
0	7.26± 1.41	7.19± 1.41	8.57± 1.73	7.66± 1.75	8.46± 1.75
14	7.84± 1.66	6.39± 1.45**a	11.46± 2.64**b	8.46± 1.94**b	9.21± 2.37**b
28	8.39± 1.65	6.91± 1.24**a	11.75± 2.76**b	8.66± 2.25-**b	10.21± 2.36**b

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- Cystone standard control (100mg/kg)

GP_4 - *VC* (200mg/kg); **GP_5** – *VC* (400mg/kg)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were significantly different from normal control (GP_1) at P< 0.01
- **(b) Values were significantly different from Lithiatic control (GP_2) at P<0.01

Figure 18: LITHOTRIPTIC ACTIVITY



EFFECT ON URINARY BIOCHEMICAL PARAMETERS ON THE DAY 14

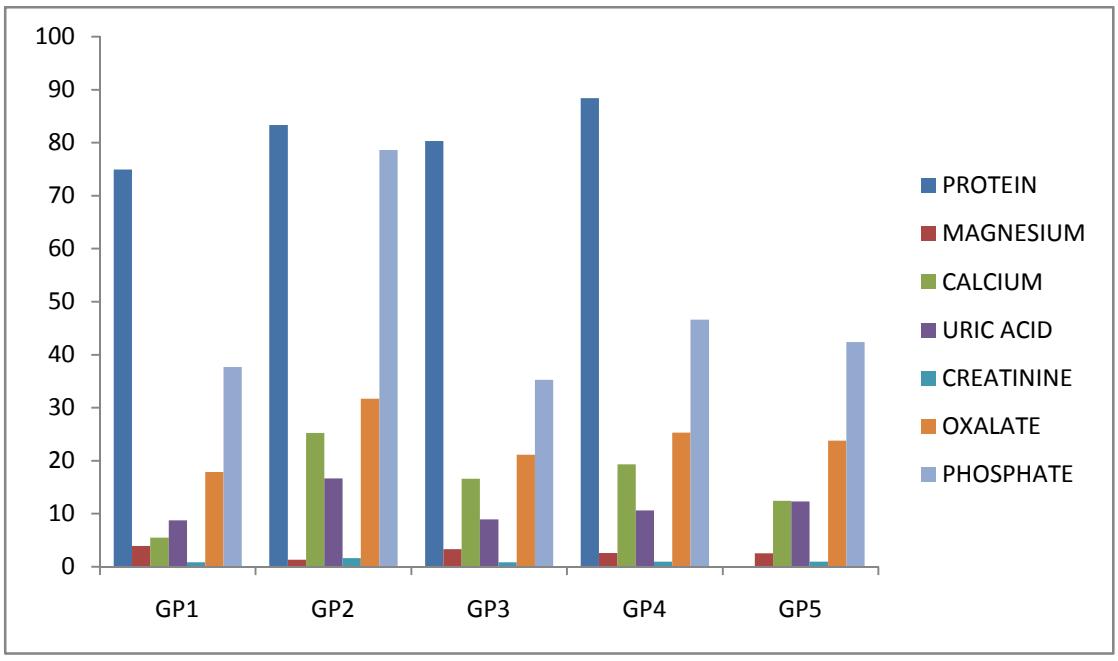
Table No: 28

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	74.96± 1.73	3.95± 0.54	5.49± 0.76	8.76± 0.88	0.82± 0.13	17.85± 1.54	37.70± 2.78
GP_2	83.31 ± 4.53**(a)	1.34 ± 0.24**(a)	25.25± 1.90**(a)	16.65 ± 1.54**(a)	1.64 ± 0.18**(a)	31.70 ± 3.25**(a)	78.65 ± 4.30**(a)
GP_3	80.34± 2.85**(b)	3.34 ± 0.60**(b)	16.63 ± 0.45**(b)	8.92 ± 0.83**(b)	0.82 ± 0.14**(b)	21.15 ± 1.76**(b)	35.25 ± 2.55**(b)
GP_4	88.38 ± 3.95 **(b)	2.60 ± 0.32**(b)	19.30 ± 2.15**(b)	10.65 ± 0.93**(b)	0.96 ± 0.12**(b)	25.32 ± 2.68**(b)	46.60 ± 3.75**(b)
GP_5	86.52 ± 3.74**(b)	2.56 ± 0.55**(b)	12.42 ± 0.83**(b)	12.34 ± 0.63**(b)	0.94 ± 0.14**(b)	23.80 ± 2.54**(b)	42.40 ± 3.26**(b)

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- Cystone standard control (100mg/kg)
GP_4 - *VC* (200mg/kg); **GP_5** – *VC* (400mg/kg)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were significantly different from normal control (GP_1) at P< 0.01
- **(b) Values were significantly different from Lithiatic control (GP_2) at P<0.01

Figure 19: EFFECT ON URINARY BIOCHEMICAL PARAMETERS ON THE DAY 14



EFFECT ON URINARY BIOCHEMICAL PARAMETERS ON THE 28TH DAY

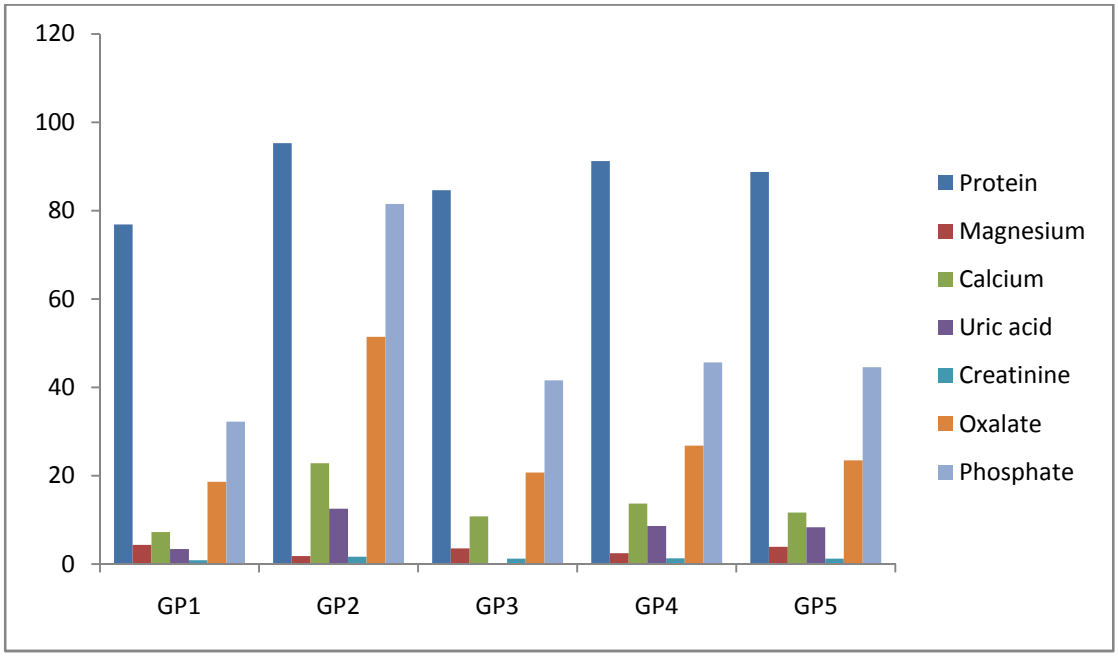
Table No: 29

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	76.85 ±3.74	4.36 ±0.40	7.25 ±0.64	3.42 ±0.73	0.90 ±0.32	18.64 ±1.41	32.25 ±3.48
GP_2	95.30 ±7.26**(a)	1.78 ±0.57**(a)	22.82 ±1.58**(a)	12.52 ±1.40**(a)	1.67 ±0.55**(a)	51.42 ±4.46**(a)	81.51 ±4.78**(a)
GP_3	84.65 ±4.84**(b)	3.55 ±0.46*(b)	10.80 ±0.49**(b)	7.22± 0.39**(b)	1.24 ±0.51**(b)	20.70 ±2.19**(b)	41.61 ±2.30**(b)
GP_4	91.20 ±5.61**(b)	2.46 ±0.40**(b)	13.70 ±1.07**(b)	8.63 ±0.41**(b)	1.28 ±0.75**(b)	26.84 ±2.65**(b)	45.68 ±3.42**(b)
GP_5	88.76 ±5.72**(b)	3.91 ±0.54**(b)	11.65 ±0.94**(b)	8.31 ±0.43**(b)	1.26 ±0.45**(b)	23.45 ±2.54**(b)	44.56 ±3.18**(b)

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- Cystone standard control (100mg/kg)
GP_4 - *VC* (200mg/kg); **GP_5** – *VC* (400mg/kg)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were significantly different from normal control (GP_1) at P< 0.01
- **(b) Values were significantly different from Lithiatic control (GP_2) at P<0.01

Figure 20: Effect on urinary biochemical parameters on the 28th day



EFFECT ON SERUM PARAMETERS ON THE 28th DAY

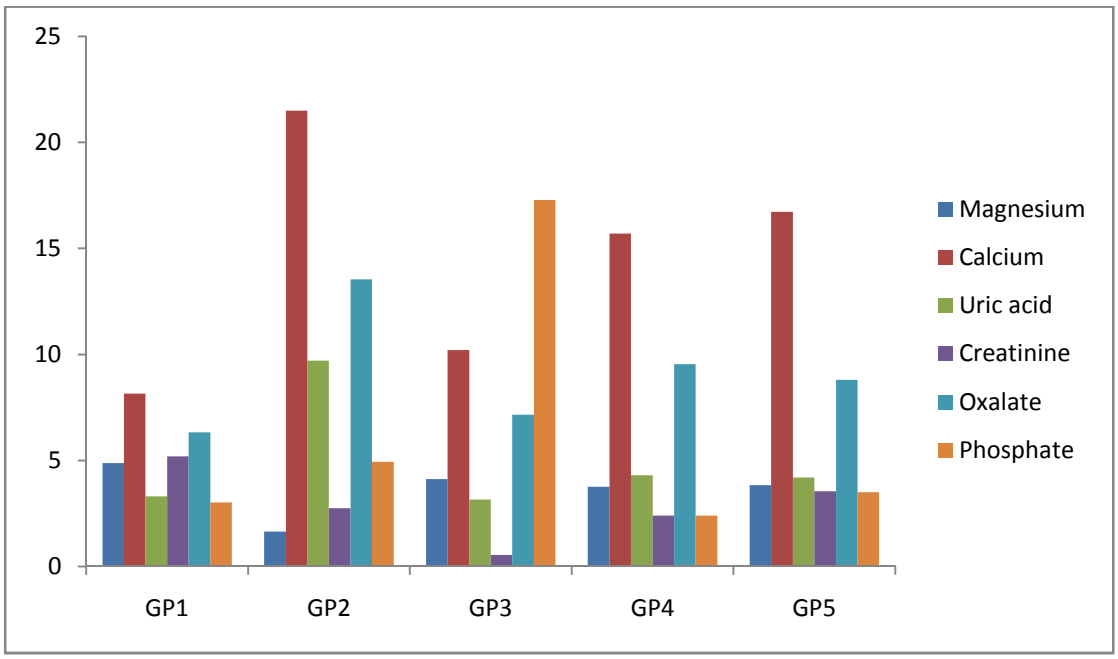
Table No: 30

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	4.88 ±0.55	8.15 ±0.19	3.30 ±0.26	5.20 ±2.15	6.32 ±0.68	3.015 ±4.250
GP_2	1.64 ±0.40**(a)	21.50 ±25.40**(a)	9.70 ±1.28** (a)	2.75 ±3.66**(a)	13.54 ±1.66**(a)	4.938 ±2.35**(a)
GP_3	4.12 ±0.33**(b)	10.20 ±1.40**(b)	3.15 ±0.45** (b)	0.54 ±0.36**(b)	7.15 ±0.32**(b)	17.28 ±1.53**(b)
GP_4	3.76 ±0.54**(b)	15.70 ±3.41**(b)	4.30 ±0.78** (b)	2.40 ±35.75**(b)	9.54 ±0.95**(b)	2.40 ±6.75**(b)
GP_5	3.84 ±0.24**(b)	16.72 ±1.23*(b)	4.20 ±0.51** (b)	3.55 ±16.22**(b)	8.80 ±0.84**(b)	3.50 ±0.87**(b)

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- Cystone standard control (100mg/kg)
GP_4 - *VC* (200mg/kg); **GP_5** - *VC*(400mg/kg)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were significantly different from normal control (GP_1) at P< 0.01
- **(b) Values were significantly different from Lithiatic control (GP_2) at P<0.01

Figure 21: EFFECT ON SERUM PARAMETERS ON THE 28th DAY



RESULT AND DISCUSSION

In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to Wister rats resulted in hyperoxaluria. Urinary concentration of the various ions investigated varied drastically, following ethylene glycol treatment.

EFFECT OF *VC* ON URINARY PARAMETERS ON DAY 14 & 28

The oxalate excretion was 24hr on day 14^{th}& 28^{th} respectively for GP_1. It increased significantly ($P < 0.001$) on day 14^{th}& 28^{th} day in GP_2 following ethylene glycol treatment. Treatment at a dose of cystone herbal tablet 100mg/kg, and VC at the dose of 200mg/kg, 400mg/kg (GP_3 to GP_5) reduced the oxalate excretion significantly to ($P<0.01$) on 14^{th} day treatment. Likewise on 28^{th} day, treatment with this *VC* reduced the oxalate excretion significantly to ($P<0.01$) in (GP_3 toGP^6) rats respectively. The results are shown in the table no: 29 & 30.

The urinary calcium excretion was increased significantly on day 14^{th} and 28^{th} day in GP_2 following ethylene glycol treatment. The calcium excretion was significantly reduced to treatment with a dose of cystone herbal tablet 100mg/kg, and VC at the dose of 200mg/kg, 400mg/kg (GP_3 to GP_5) reduce the calcium excretion significantly to on 14^{th} day treatment likewise on 28^{th} day calcium excretion was significantly reduced to s24hr ($P<0.01$) in (Gp_3 to Gp_5) rats respectively.

Likewise phosphate and creatinine excretion values gradually increased in GP_2 on the 14^{th}& 28th day. However in (GP_3 to GP_5) grouped treated animals these elevated values were significantly reduced on 14^{th} and 28^{th} day respectively. However, regarding creatinine in (GP_3 to GP_5) these elevated values were significantly reduced on 14^{th} day and on 28^{th} day respectively.

Likewise urinary protein and uric acid concentration increased following ethylene glycol treatment in GP_2 and it reached maximum respectively on the 14^{th}& 28^{th} day. On treatment with a dose of cystone herbal tablet 100mg/kg, and VC at the dose of 200mg/kg, 400mg/kg (GP_3 to GP_5) the protein and uric acid excretion was restored to near normal limits in (GP_3 to GP_5) for protein on 14^{th}day and on 28^{th} day ($p<0.001$) and for uric acid on 14^{th} day and on 28^{th} day ($P<0.01$).

In GP_2 lithiatic control rats, the magnesium level in urine gradually decreased following ethylene glycol treatment on the 14^{th} & 28^{th} day. Subsequent administration of the *VC* and cystone herbal tablets enhanced the magnesium excretion significantly on 14^{th} day & 28^{th} day.

EFFECT OF *VC* ON SERUM PARAMETERS ON DAY 28

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased significantly in GP_2 (Lithiatic control) following ethylene glycol treatment, treatment with a dose of cystone herbal tablet 100mg/kg, and VC at the dose of 200mg/kg, 400mg/kg (GP_3 to GP_5) reduce the all above mentioned parameters significantly. On the contrary the magnesium levels were decreased significantly in GP_2 (Lithiatic control) following ethylene glycol treatment**.** After treatment with a dose of cystone herbal tablet 100mg/kg, and VC at the dose of 200mg/kg, 400mg/kg (GP_3 to GP_5) the magnesium level was restored near to normal levels.

INTERPRETATION

The result shows that the VC at a dose of 200, 400mg/kg and cystone herbal tablet at a dose of 100 mg/kg increased elimination of oxalate, calcium, phosphate, magnesium, uricacid, creatinine which decreased circulating level of those minerals and as prevent calculi formation of kidneys.

The test drug VENKARA CHUNNAM prevent calculi formation of kidney. so the test drug is responsible for antilithiatic activity.

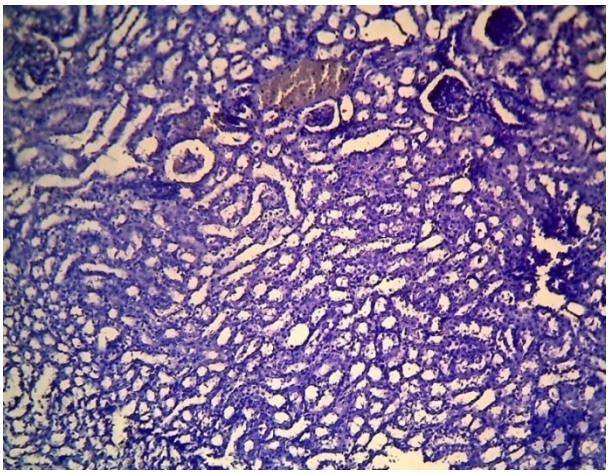
LITHOTRIPTIC ACTIVITY

Effect on urinary output in urolithiasis induced rats

HISTO PATHOLOGY

Figure:22

Name : **LITHOTRIPTIC - NORMAL**



Clinical Details:

LG -1 Normal control

Clinical Diagnosis :

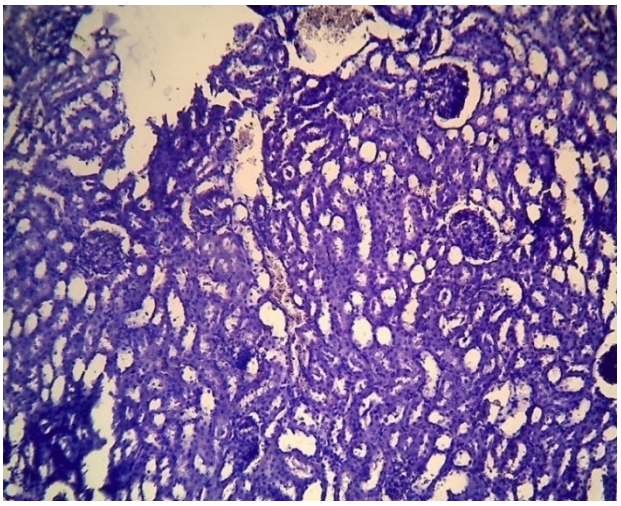
Macroscopic :

Microscopic:

Normal renal tissue is seen.

HISTOPATHOLOGY

Name : **LITHOTRIPTIC - TOXIC**



Clinical Details:

LG – 2 (Toxic)

Clinical Diagnosis :

Macroscopic :

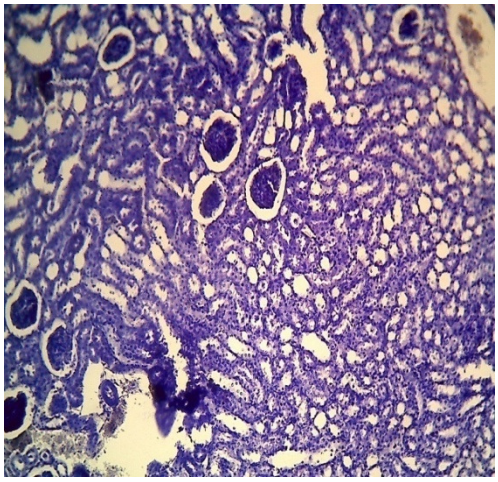
Microscopic:

Toxic renal tissue seen.

crystals/stones or calcification.

HISTOPATHOLOGY

Name : **LITHOTRIPTIC – STD/ CONTROL**



Clinical Details:

LG – 3 Standard/control

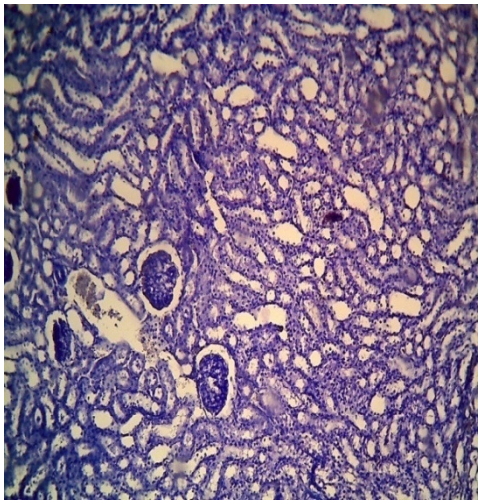
Clinical Diagnosis :

Macroscopic :

Microscopic:

Normal renal tissue seen

Name : **LITHOTRIPTIC – LOW DOSE**



Clinical Details:

LG -4 ; low dose

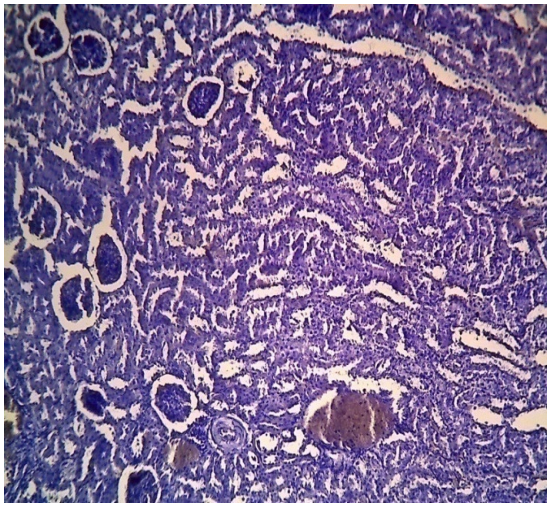
Clinical Diagnosis :

Macroscopic :

Microscopic:

Normal renal tissue seen.

Name : **LITHOTRIPTIC – HIGH DOSE**



Clinical Details:

LG – 5; High dose

Clinical Diagnosis :

Macroscopic :

Microscopic:

Normal renal tissue seen.

Histopathology:

Group I: The normal section of renal tissue seen

Group II: The renal section showing the damaging cells seen.

Group III: The normal renal tissues are seen compare to damaging renal tissue

Group IV: The normal renal tissues are seen nearest compare with the standard drug.

Group V: The normal renal tissues are seen compare to low dose of VC.

2. DIURETIC ACTIVITY

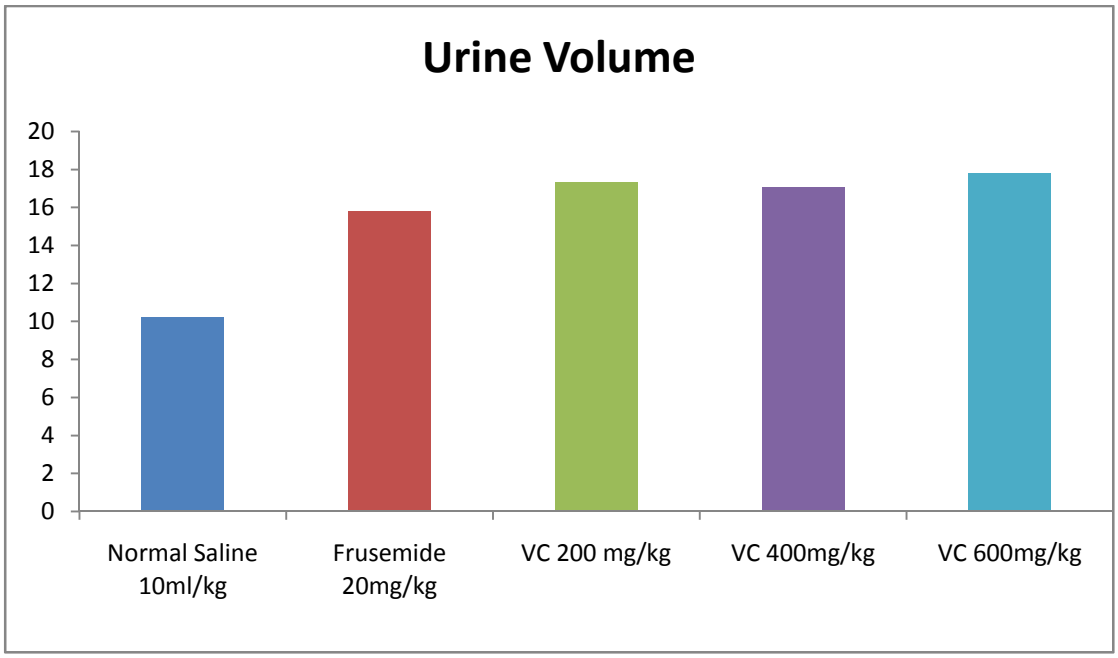
Diuretic activity of *VC* (urine Volume) in 24 h

Table No: 31

Group	Treatment	Urine Volume
I	Normal saline 10ml/kg	10.23±0.56
II	Frusemide 20mg/kg	15.80±0.98
III	VENKARA CHUNNAM 200mg/kg	17.33±0.73**
IV	VENKARA CHUNNAM 400mg/kg	17.1±0.71**
V	VENKARA CHUNNAM 600mg/kg	17.81±0.93**

Values are Mean ± SEM, n=6, **p<0.01.

Figure 23: Diuretic activity of *VC* (urine Volume) in 24 h



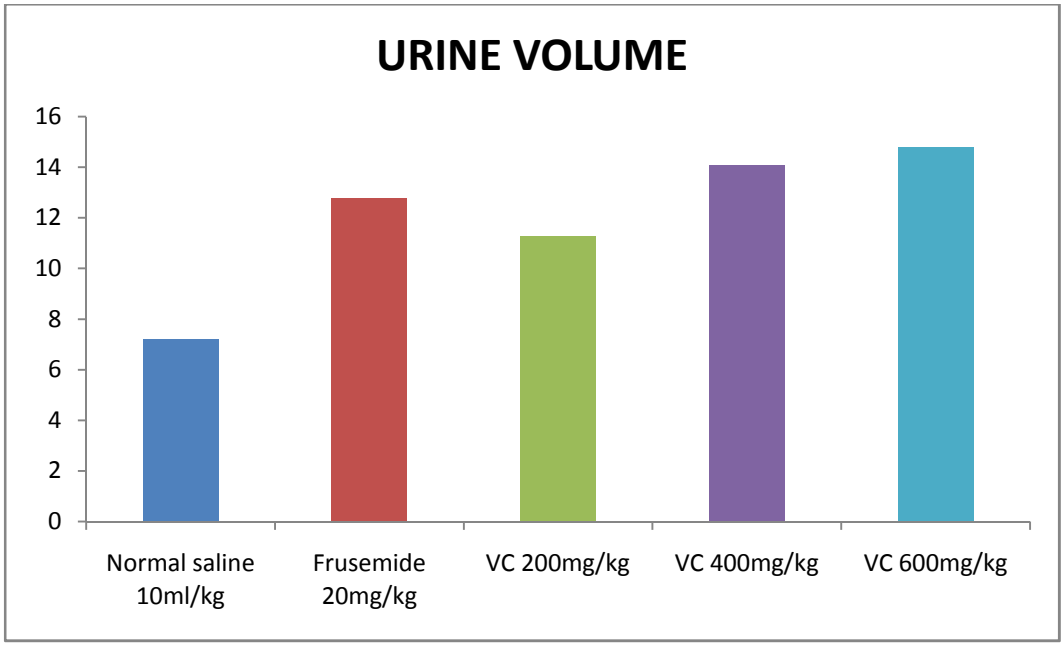
Natriuretic activity of VC

Table No: 32

Group	Treatment	Urine Volume
I	Normal saline 10ml/kg	7.20±0.53
II	Frusemide 20mg/kg	12.77±0.95
III	VENKARA CHUNNAM 200mg/kg	11.27±0.67**
IV	VENKARA CHUNNAM 400mg/kg	14.1±0.68**
V	VENKARA CHUNNAM 600mg/kg	14.78±0.90**

Values are Mean ± SEM, n=6, **p<0.05, **p<0.01.NS – Not significant.

Figure 24: Natriuretic activity of VC



Results:

Table -32 shows the urine volume collected in 24 h for all the groups. It is evident that the *VC* treated groups excreted more urine than the control groups. The *VC* at 200, 400 and 600 mg/kg exhibited comparable effect with that of the reference drug Frusemide 20 mg / kg and the results were statistically significant Table -33 shows the sodium and potassium content of the urine for all groups. The amount of Sodium excreted was increased for Frusemide treated group; statistically significant rise in Na+ excretion was also noticed for *VC* treated groups. The potassium content excreted in the urine was statistically insignificant for all the groups. The Natriuretic effect was calculated by employing the formula Na+ / K+. It was found that the *VC* treated groups possess favorable Natriuretic effect. The present study showed that the *VC* significantly increases the urine output and excretion of urinary sodium and had no effect on the urinary potassium excretion. Diuretics have two separate connotations; increase urinary par se and net loss of solute (i.e. electrolyte) and water (i.e. saluretic). These two processes are involved in the suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream and a consequence; promote the formation of urine . An attempt to extrapolate the diuretic action of plant extract from rats to man using the activity of Frusemide in the organism as a guideline has been reported.

INTERPRETATION:

The results clearly shows that the *VC* at doses of 200, 400 and 600 mg / kg produced significant dose dependent increase in urinary excretion and urinary sodium loss but no effect on urinary potassium loss with respect to control and standard drug treated groups. The data demonstrates that the *VC* has diuretic effect, Natriuretic effect but no potassium sparing effect and is as potent as Frusemide.

3. ANTI-SPASMODIC ACTIVITY (Smooth muscle relaxant)

Dose Response Relationship Observations of Acetylcholine

Table No: 33

S.No	Concentration/dose	Acetylcholine
		Response (cm)
1	0.1 ml	3.4 cm
2	0.2 ml	4 cm
3	0.4 ml	4.3 cm
4	0.8 ml	4..3 cm
5	1.6 ml	5.7 cm

Dose Response Relationship Observations of Atropine

Table 34

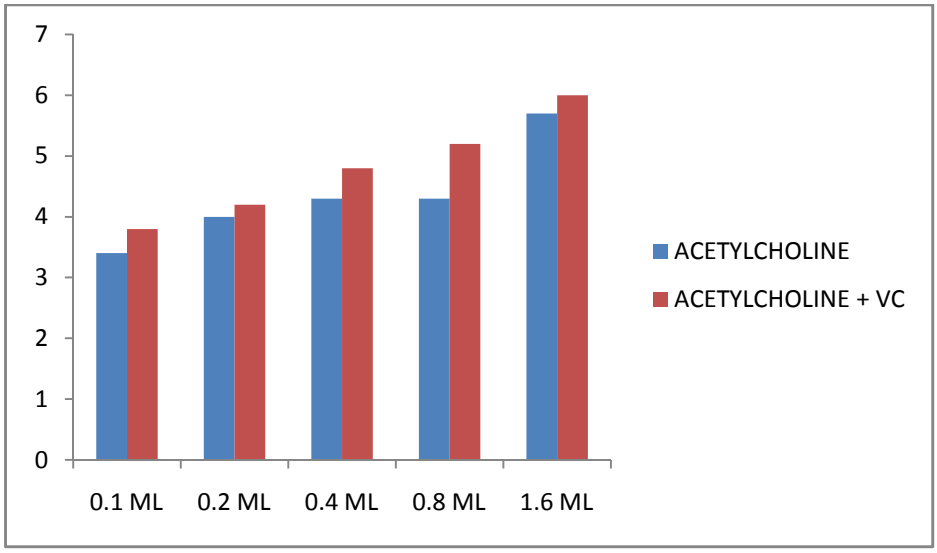
Si.No	Concentration/dose	atropine
		Response (cm)
1	0.1 ml	-
2	0.2 ml	-
3	0.4 ml	-
4	0.8 ml	-
5	1.6 ml	-

Comparative Dose Response of Acetylcholine followed by *VC*

Table No: 35

Sl. No	Treatment	Dose(ml)	response	% of response
1	Acetylcholine	0.1 ml	3.4 cm	
2		0.2 ml	4 cm	
3		0.4 ml	4.3 cm	
4		0.8 ml	4..3 cm	
5		1.6 ml	5.7 cm	
6	Acetylcholine ***+VC***	0.1 ml+0.1 ml	3.8 cm	
7		0.2 ml+0.2 ml	4.2 cm	
8		0.4 ml+0.4 ml	4.8 cm	
9		0.8 ml+0.8 ml	5.2 cm	
10		1.6 ml+1.6 ml	6 cm	

Figure 25: Comparative Dose Response of Acetylcholine followed by ***VC***



RESULTS:-

Effect of Acetylcholine on excised rat ileum reflected an increase in spasmodic activity (response) with an increase in dose.

INTERPRETATION:

From the present study results it was observed that acetylcholine (Ach) alone cause contraction of excised rat ileum but when acetylcholine was given in presence of ***VC*** there was a marked decrease in contraction of ileum was observed. This revealed that ***VC*** possess a high degree of anti-spasmodic (smooth muscle relaxant) activity by blocking cholinergic receptors.

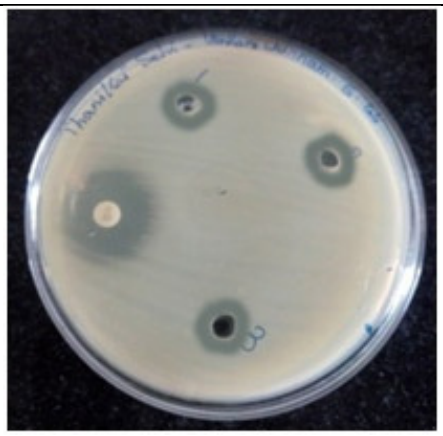
MICRO BIOLOGICAL ANALYSIS

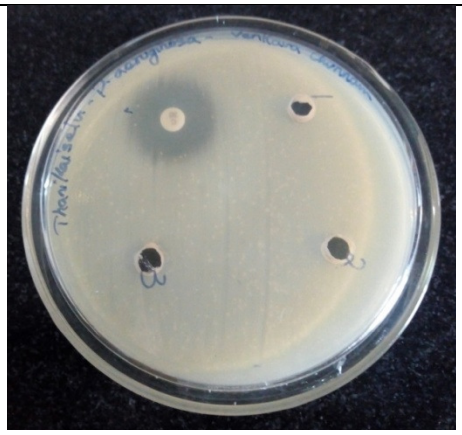
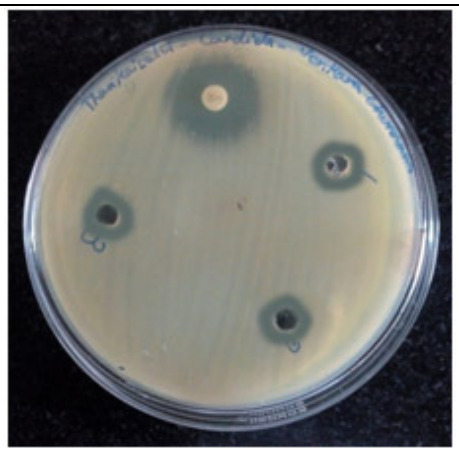
Antimicrobial Activity

Table no. 36

S.No.	Test Pathogens	Result	Zone of Inhibition (mm) at 30µl	
			Positive Control (Gentamycin)	Size of Inhibition
1.	*Escherichia coli*	Sensitive	20 mm	18 mm
2	*Klebsiella pneumoniae*	Sensitive	22 mm	16 mm
3	*Staphylococcus aureus*	Sensitive	21 mm	11 mm
4	*Pseudomonas aeruginosa*	Resistant	17 mm	17 mm
5.	*Salmonella typhi*	Sensitive	19 mm	13 mm

Figure no : 26 **Anti – Microbial Activity of *VENKARA CHUNNAM***

S.No.	Bacterial Pathogens	Plates
1.	*Escherichia coli*	
2.	*Staphylococcu aureus*	

3.	*Klebsiella sps.*	
4.	*Pseudomonas aeruginosa*	
5.	*Candida sp*	

Results and Discussion:

The good antibacterial activity of herbal medicines implies that the antimicrobial compounds present in herbal medicines are possibly controlling the microbial activity. Herbal medicines showed varying degrees of *in vitro* antibacterial activity against test bacteria.

Both Gram positive and Gram negative bacteria *E.coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Salmonella typhi* were found to be high sensitive to herbal medicines when compared to the standard drug Gentamycin (Broad

spectrum). The herbal drug **VENKARA CHUNNAM** exhibited broad spectrum activity against bacterial pathogens at 100mg / ml concentration of the drug.

From these results, it is accomplished that this study would lead to the establishment of several important compounds that have to be used to formulate new, different and more potent antimicrobial drugs of natural origin. However, further studies are required to screen the biologically active compounds and to evaluate the efficiency of this compound against pathogenic microorganisms associated with various human diseases.

INTERPRETATION:

- From the above results the test drug VC is sensitive against *Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Salmonella typhi* when compared to the standard drug (Gentamycin 100 mcg) which was evident from the zone of inhibition.
- As the above bacterial organisms reponsible for pneumonia, bronchitis, pharyngitis, diarrhoea, typhoid fever, headache, urinary tract infectiona nd skin infections.
- Our results confirmed the traditional use of VC has anti-microbial activity.

7. SUMMARY

1. The trial drug *VENKARA CHUNNAM* is a herbo mineral formulation selected from the Siddha Literature *Anuboga vaithiya navaneetham, part 3 page no 25, 26,* Second Edition - 2002, Hakim P. Mohamed Abdulla Sahib, published by thamarai noolagam chennai 26 for the validation of safety, efficacy and therapeutic potency on *Kalladaippu* for its lithotriptic, diuretics and antispasmodic activities.
2. The raw drug *Venkaram, Uppilangodi, Muurungai pattai* were identified and authenticated by Gunapadam department, Govt. Siddha Medical College, Palayamkottai, Tamil Nadu.
3. A review of the literatures and lateral research works reveals that all the ingredient of *VENKARA CHUNNAM* traditionally used in treating *kalladaippu.*
4. Physicochemical analysis shows determination of moisture content it was within acceptable range. It indicates the longer shelf life period.
5. Biochemical analysis shows the presence of calcium, sulphate, chloride, ferrous iron, phosphate and unsaturation. Sulphate has antibacterial activity, Chloride has diuretic activity, Ferrous iorn has antioxidant activity.
6. Phyto-chemicals in the drug of *Venkara Chunnam* using various test and the results are the presence of Alkaloids, carbohydrates and glycosides, tannins, and absence of phytosterols, flavonoids, fixed oil, fat, proteins, saponins and lignin. Alkaloids have antimicrobial activity. Glycosides inhibit diseases producing microorganisms. Tannins has antioxidant property.
7. SEM analysis of these drugs shows the smaller particles are rounded in shapes and sizes are in the range from 10 micron to 5 micron. The smaller size of the particles enables better absorption which denotes that the trial drug could have potent drug delivery.
8. FTIR analysis of this drug revealed the presence of Carboxylic acid, alkenes, alcoholic groups, aliphatic amines, primary and secondary amines, alkyl halides, cyloalkane, esters and ethers. Alcoholic acids, act as antimicrobial and antiseptic agents prevent the formation of renal calculi (infective cause), carboxylic acid substance has antimicrobial, anticoagulant activity. Amines are good pain relievers. Alkenes groups protect against

bacteria and fungi.Alkyl halides used as anesthetics and antiseptic agents. Esters group is produced a diuresis and natriuresis. So result of a direct tubular action together with a pressure diuresis. Ethers increases the flow of urine, It is a best diuretic action. A diffusible stimulant and antispasmodic used chiefly in colic.

9. ICP-OES analysis of VC shows presence of K, Mg, P, Na, B, Fe, Ca and heavy metals like As, Hg, Cd, Pb, Cu, Ni, Al were below detectable level. The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits. Presence of potassium, relaxes the smooth muscles, so it have smooth muscle relaxant acticity. Presence of magnesium inhibits the formation of calcium oxalate crystals, it also inhibit the stone formation by inhibition of growth of crystals as well as aggregation. Sodium regulates the acid base balance of the body fluids. Sodium is required for the maintenance of the osmotic pressure.
10. This XRD finger print shows both the similarities and difference of the sample successfully and is a valuable primary tool for checking the quality control of above mineral medicines. Modern techniques are necessary to standardize and bring out high quality herbal product owing to their complex nature. The different peak shows the presence of minerals in the sample.
11. The pharmacological study revealed that trial drug *VENKARA CHUNNAM* possesses significant lithotriptic activity, Increased elimination of oxalate, calcium, phosphate, magnesium, uric acid, creatinine which decreased circulating level of those minerals and as prevent calculi formation of kidneys. So the test drug are responsible for antilithiatic activity.
12. Significant dose dependent increase in urinary excretion and urinary sodium loss but no effect on urinary potassium loss with respect to control and standard drug treated groups. The data demonstrates that the *VENKARA CHUNNAM* has diuretic effect and possesses a high degree of antispasmodic activity.
13. Microbiological analysis of VENKARA CHUNNAM showed that it is moderate sensitive against staphylococcus aureus, pseudomonus aeruginosa, and E.coli. so it has antimicrobial activity.
14. The trial drug *Venkara chunnam* has sweet and astringent taste. The deranged pitha humour, decreases the excretion of urine and increases the urine

concentration which resulted in *kalladaippu.* So these tastes of the trial drug neutralize the pitha humour, increase the excretion of urine, decreases the concentration of urine and prevent the formation of *kalladaippu*.

15. The acute toxicity result shows the test drug VC does not produce any toxic signs and mortality up to its maximum dose level of 2000 mg/animal body weight in Wistar albino rats. Morphological characters like changes in skin, eyes, fur, and nose appeared normal and behavioural changes are normal. According to OECD guidelines, LD_{50} dose up to 2000mg/kg of the drug the test drug *VENKARA CHUNNAM* is a safe herbo mineral drug and can be used for long time administration.
16. Finally all the parameters and histopathological studies results revealed the drug was safe in rats. Acute oral toxicity of VC observed no toxicity. Acute and sub-acute toxicity study of VENKARA CHUNNAM represents nontoxic and safe drug in wistar albino rats. biochemical parameters and histopathology results it can be concluded that the dose level of VC 100 mg mentioned in the Siddha Literature "*Pathartha Gunavilakkam"* is the safety dose for human consumption. So the test drug of VC hope fully use for human trails.
17. Acute and sub-acute toxicity were carried out in wistar albino rats according to OECD guidelines (423,407). This drug has no acute toxicity as there was no mortality seen in sub-acute toxicity is carried by repeated dose of test drug for 28 days, which reveals there are no behavioural changes, morphological changes, haematological changes, biochemical changes observed in rats.

8. CONCLUSION

The trial drug ***VENKARA CHUNNAM*** was selected for the elaborate study of its efficacy on *Kalladaippu*. From the literature review, physiochemical, biochemical, Phytochemical, Instrumental analysis, pharmacological, microbiological analysis It has been concluded that *VENKARA CHUNNAM* has got a good Lithotriptic (Anti lithiatic), Diuretic, Anti – spasmodic activities and its efficacy is scientifically validated.

9. FUTURE SCOPE

The trial drug ***VENKARA CHUNNAM*** has its own potency in treating urolithiasis in animal model which has been established in this study. However, the mechanism of action by which *VENKARA CHUNNAM* produced its effect on the urolithiasis in experimental animal models and also multicenter clinical trials are required to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of urolithiasis.

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